VOLUME 29 FEBRUARY 1951 NUMBER 1

# Canadian Journal of Zoology

*Editor:* E. HORNE CRAIGIE

NOTE—This Journal prior to January, 1951, was Section D (Zoological Sciences) of the Canadian Journal of Research.

*Published by* THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

## CANADIAN JOURNAL OF ZOOLOGY

This was formerly *Section D, Canadian Journal of Research.* The change to the new name took place January 1, 1951. The CANADIAN JOURNAL OF ZOOLOGY is published six times annually.

The CANADIAN JOURNAL OF ZOOLOGY is published by the National Research Council of Canada under the authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. Matters of general policy are the responsibility of a joint Editorial Board consisting of members of the National Research Council of Canada and the Royal Society of Canada.

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# ON THE NEMATODE GENUS *RHABDOCHONA* RAILLIET, 1916 (NEMATODA: SPIRUROIDEA)[1]

BY L. P. E. CHOQUETTE[2]

## Abstract

The systematic position of the genus *Rhabdochona* Railliet, 1916 and its diagnosis is reviewed. The genus comprising 33 species has a world-wide distribution and has been found, with one exception, in fish. Species from fish in North America are discussed and *R. cascadilla* Wigdor, 1918 is redescribed; *R. laurentiana* Lyster, 1940 is considered to be identical with the latter. A new species, *R. milleri*, is described. A host list and the geographical distribution of the 33 species of *Rhabdochona* so far recorded are given.

## (1). The Systematic Position of the Genus *Rhabdochona* Railliet, 1916

The family Thelaziidae was created by Railliet (46) in 1916 to include a number of genera of the superfamily Spiruroidea parasitic in mammals, birds, and fishes with *Thelazia* Bosc., 1819, as the type genus. In addition Railliet transferred to this family the genera *Ceratospira* Schneider, 1866, *Cystidicola* Fischer v. Waldheim, 1798, and *Oxyspirura* Drasche, 1897, and the four new genera *Galeiceps*, *Schistorophus*, *Serticeps*, and *Rhabdochona*.

Subsequently, the family Thelaziidae was amended to some extent by Baylis and Daubney (7), by Yorke and Maplestone (60), and by Chitwood and Wehr (12).

In 1928, Travassos *et al.* (54) created the subfamily Rhabdochoninae which they placed in the family Spiruridae, to include the genera *Rhabdochona*, *Cystidicola*, and *Spinitectus* Fourment, 1883.

These authors considered as belonging to this subfamily, those forms parasitic in fish which are characterized by the presence of a bicuspid mouth with lips pronounced in some cases and rudimentary in others and of a pharynx having the shape of an elongated chitinized tube, slightly widening in its anterior part; two unequal and dissimilar spicules and the absence of a gubernaculum; and by the presence of numerous preanal and postanal papillae.

Skrjabin (49), in his revision of the taxonomy of the Spirurids parasitic in fish, commenting on the opinion of Travassos and his colleagues, considered that

[1] *Manuscript received July 5, 1950.*

*Contribution from the Institute of Parasitology, Macdonald College (McGill University), Macdonald College, P.O., P.Q., with financial assistance from the National Research Council of Canada.*

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[*The December issue of Section D (Can. J. Research, D, 28: 293-331. 1950) was issued Dec. 1, 1950.*]

the genera *Capillospirura* Skrjabin, 1924, *Metabronema* Yorke and Maplestone, 1926, and *Comephoronema* Layman, 1933 should be regarded as being related to the Rhabdochoninae as defined by Travassos *et al.* Skrjabin admitted the validity of the subfamily Rhabdochoninae as defined by Travassos and his colleagues but was of the opinion that it should not be placed in the family Spiruridae Cerley, 1885, because of the morphological differences from *Spirura* Blanchard, 1849, the type genus of the family. He proposed, accordingly, the creation of a new family, Rhabdochonidae, to include those spirurids of fishes characterized by a thin delicate body, a mouth opening into a funnel-shaped oral cavity, a thin, elongated pharynx, the presence of cervical papillae, a clear-cut double oesophagus, unequal and dissimilar spicules, and numerous postanal papillae. This family Skrjabin divided into three subfamilies: Rhabdochoninae, Cystidicolinae, and Spinitectinae. Members of the subfamily Rhabdochoninae of Skrjabin have a smooth cuticle and eggs without polar filaments; in this subfamily he placed *Rhabdochona* and *Sterliadochona* Skrjabin, 1946. In the Cystidicolinae the eggs are provided with polar filaments; the subfamily includes *Cystidicola*, *Capillospirura*, *Metabronema*, *Comephoronema*, and *Pseudocystidicola* Layman, 1933. In the Spinitectinae the body is spinous; it contains the single genus, *Spinitectus*.

One of the advantages of the classifications proposed by Travassos *et al.* (54) and Skrjabin (49) is the grouping of related forms parasitic in similar hosts, that is, of spirurid worms in fish hosts, in spite of the fact that it unites species which Chitwood and Wehr (12) have shown to have evolved along different lines. On the other hand, the writer is not willing at present to admit the validity of some of the characteristics used by Skrjabin (49) in his differentiation of the various families, for example, polar filaments are present in some species of *Rhabdochona* and lacking in some species of *Cystidicola*. In the writer's opinion, the family Rhabdochonidae Skrjabin, 1946, should consist of only two subfamilies: Rhabdochoninae and Spinitectinae. The former would include those spirurids of fish with a smooth cuticle, belonging to the genera *Rhabdochona*, *Sterliadochona*, *Cystidicola*, *Capillospirura*, *Metabronema*, *Comephoronema*, and *Pseudocystidicola*. The second subfamily would include only the genus *Spinitectus*.

## (2) Diagnosis of the Genus *Rhabdochona*

*Rhabdochona* Railliet, 1916

Type: *Dispharagus denudatus* Dujardin, 1845 (18) designated by Railliet (46).

The known species of this genus are, except in one case, parasitic in fish hosts, Pearse (44) having described a species, *R. uca*, from the fiddler crab. The main characters used in the diagnosis of *Rhabdochona* are the morphology of the cephalic structures, the anterior part of the alimentary canal and the posterior extremity of the body, and, in the male, the number and arrangement

of pre- and postanal papillae, the absence of caudal alae, the relative size of the spicules; in the female, the position of the vulva, the size of the eggs, the presence or absence of filaments on the eggs are characters of diagnostic value.

In their descriptions of the genus *Rhabdochona* Railliet (46), Yorke and Maplestone (60), and Baylis and Daubney (7) mention the presence of lips; Chitwood and Wehr (12) have shown lips to be absent in the material studied by them. The writer's observations and those of Gendre (25) are in accord with those of Chitwood and Wehr in this respect. One of the characteristic morphological characters of *Rhabdochona*, is the funnel-shaped prostom supported by longitudinal ribs protruding anteriorly as teeth. Chitwood and Wehr (12) in their diagnosis of the genus give the number of these teeth as 10 or 12. However, this number may be greater as, for instance, in the form described as *R. laurentiana* by Lyster (42), *R. acuminata* (Molin, 1860), Gendre, 1921 (25), and *R. kidderi* Pearse, 1936 (Chitwood (11) ), and others (Gustafson (27) ), where the number of teeth is 14; it may be smaller as in *R. paski* Baylis, 1926 (6). A review of the literature shows that this valuable specific character has been overlooked in many cases, probably owing to the difficulty of preparing specimens for study. In addition to these teeth, projections have been shown to exist at the base of the prostom in some species.

In the diagnosis of the genus *Rhabdochona* the spicules of the male are given as unequal in length and dissimilar. Fujita (21, 23) in his description of *R. salvelini* states that the two spicules are equal in length and thus amended the diagnosis of the genus. However, examination of Fujita's illustration of the posterior extremity of the male (Fig. 3 (21, 23) ), shows that the spicules are definitely unequal in length with his "accessory spine" corresponding to the short spicule. Weller (56) in his description of *R. ovifilamenta* reports and illustrates the presence of a strongly chitinized gubernaculum. The writer had the opportunity of examining the mounted specimen used by Weller in the preparation of his illustration and, in the writer's opinion, what appears to be a "gubernaculum" is an optical effect caused by a bend in the spicule. There are only two records of species of this genus in which the eggs are provided with polar filaments: *R. ovifilamenta* Weller, 1938 (56) and *R. cotti* Gustafson, 1949 (27).

The genus *Rhabdochona* can thus be defined: mouth without lips or pseudolabiae, circumoral membrane bounding a funnel-shaped prostom supported by longitudinal thickenings projecting anteriorly as teeth, the number of which is variable, mesostom long and narrow. Oesophagus composed of two unequal parts. Male: several pairs of pre- and postanal papillae; no caudal alae, spicules unequal and dissimilar; gubernaculum absent; tail conical, pointed or provided with a chitinous process. Female: uteri opposed; vulva about middle of body; eggs elliptical, embryonated, with or without polar filaments. Parasites of aquatic vertebrates.

## (3) The Genus *Rhabdochona* in North America

From North America six species belonging to the genus *Rhabdochona* have been described from fish hosts. In addition, during the course of this study, a new species was found and described as *Rhabdochona milleri*. These species, the type host, and locality, are shown in Table I.

TABLE I

SPECIES OF THE GENUS *Rhabdochona* RECORDED IN NORTH AMERICAN FISH HOSTS

| Species | Type host | Locality |
|---|---|---|
| R. *cascadilla* Wigdor, 1918 | *Semotilus atromaculatus atromaculatus* *Notropia cayuga* | Cayuga Lake, New York State, U.S.A. |
| *R. ovifilamenta* Weller, 1938 | *Perca flavescens* | Big Stone Bay, Mackinaw Straits, Michigan State, U.S.A. |
| *R. laurentiana* Lyster, 1940 | *Catostomus catostomus* | Lake Commandant, P.Q., Canada. |
| *R. decaturensis* Gustafson, 1949 | *Aplodinotus grunniens* | Lake Decatur, Illinois, U.S.A. |
| *R. cotti* Gustafson, 1949 | *Cottus cognatus* | Spokane, Washington, U.S.A. |
| *R. pellucida* Gustafson, 1949 | *Pteichocheilus oregonensis* | Davis Lake, Washington, U.S.A. |
| *R. milleri* nov. sp. | *Moxostomum aureolum* | Ottawa River, Pointe-au-Chene, P.Q. |

While there is only one record of *R. ovifilamenta* (Weller (56) ), and two of *R. laurentiana* (one by Lyster (42) and one by the writer (13), who records it from the brook trout (*Salvelinus fontinalis*) ), a species identified as *R. cascadilla* Wigdor, 1918, has been recorded by Bangham (2), Bangham and Hunter (3), Bangham and Venard (5), and by Fischthal (19, 20), in a score of fish hosts from both the United States and Canada. Some of the material collected by Bangham and his associates and by Fischthal has been made available for study. In addition to lending some of the material upon which published records of *Rhabdochona* were based, Dr. Bangham also kindly loaned to the author material which he had collected from various hosts from different localities in the State of Wisconsin, U.S.A. This material, and some collected by the writer, was studied and species identified. Those identified as belonging to the genus *Rhabdochona* are reported here and included in the list of hosts and geographical distribution.

From Canada, Bangham (1) and Bangham and Venard (5) report the presence of unidentified species of *Rhabdochona* in *Catostomus commersonii*,

*Ameirus nebulosus*, *Perca flavescens*, *Lepomis gibbosus*, *Pimephales promelas*, and *Coregonus clupeaformis*. Only specimens from the first two of these hosts were available for study; these were found to be *R. cascadilla*.

Bangham and Hunter (3) report also the presence of unidentified species of *Rhabdochona* in *Fundulus diaphanus menoma*, *Lepibema chrysops*, *Helioperca incisor*, and *Ambloplites rupestris* from Lake Erie, U.S.A. Only some specimens from the top minnow, *Fundulus diaphanus menoma*, were available for study and these were found to belong to another genus than *Rhabdochona*. Bangham (2) reports the presence of a larval form of *Rhabdochona* in the digestive tract of *Rhinicthys atratulus meleagridis* from the Brule River, Wisconsin; this is probably the larval stage of *R. cascadilla* shown to be prevalent in this locality (19, 20).

*Rhabdochona milleri* nov. sp. (Fig. 1).

From the redhorse sucker, *Moxostoma aureolum*, forms belonging to the genus *Rhabdochona* were found by Dr. M. J. Miller of this Institute and given to the author for identification. These prove to represent a new species which is named in Dr. Miller's honor.

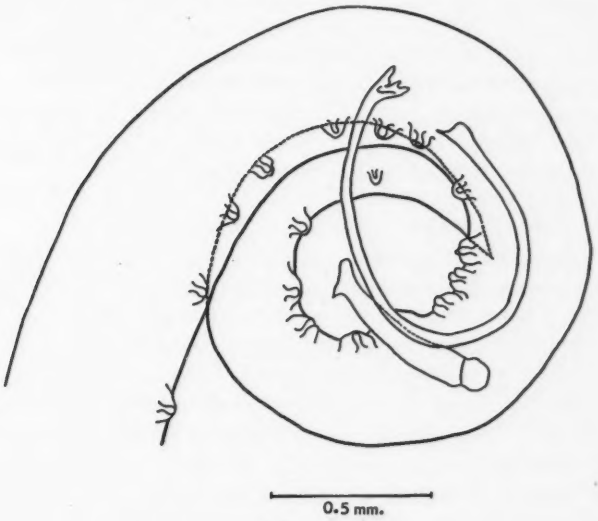


FIG. 1.

The material upon which this new species is described consists of only one male and of two females. The male measures 13.39 mm. and the females up to 17.9 mm. in length. The funnel-shaped prostom is about 25$\mu$ in length and is guarded anteriorly by teeth, the exact number of which could not be determined distinctly even in *en face* view. The mesostom is from 100 to

130$\mu$ long. The excretory pore is from 300 to 370 $\mu$ from the anterior extremity, a little distance behind the nerve ring, which is placed anteriorly on the first portion of the oesophagus.

The oesophagus is from 3.7 to 4.3 mm. long and in this particular case is longer in the male specimen than in the two female specimens examined. The anterior portion is much shorter than the posterior one, their respective lengths being in the ratio of 1 : 11–12 in the female and 1 : 10 in the male. The anus is from 300 to 400$\mu$ from the tail end which is pointed and spined.

The tail in the male (Fig. 1) is provided with 11 pairs of preanal and with five pairs of postanal papillae. The two spicules are unequal; the longest one bifurcates at its distal extremity and measures 598$\mu$, while the short one is 202$\mu$ long. In the female, the vulva is approximately equatorial in position and the eggs measure 28 to 30$\mu$ by 19 to 20$\mu$.

*R. milleri* and *R. kidderi* (45) are the only species of the genus *Rhabdochona* described in which there is a spicular bifurcation. The number of papillae, the size of the spicules, and the ratio of their length is sufficient to distinguish these two species.

Type host: *Moxostoma aureolum.*

Location: Intestine.

Locality: Ottawa River, Pointe-au-Chene, (Co. Argenteuil) Prov. of Quebec, Canada, July, 1940.

Specimens: Holotype, allotype, and one paratype: presently in the helminthological collection of the Institute of Parasitology, Macdonald College, McGill University.

*Rhabdochona cascadilla* Wigdor, 1918

For the purpose of comparison and study, attempts have been made to secure the type specimens or some other material used by Wigdor for his description of *R. cascadilla*. However, these attempts have so far been unsuccessful. More than thirty years have elapsed since Wigdor's discovery and it is rather doubtful whether this material will ever be found. (The writer takes the opportunity of drawing attention to the necessity of depositing and keeping type material in recognized collections where it will be accessible to subsequent workers.)

Some of Wigdor's data on the morphology of the nematode have been used rather uncritically by later workers in the determination of species. Thus, Spaul (50), and Lyster (42), use the length of the oesophagus as a criterion in the differentiation of *R. anguilla* and *R. laurentiana* from *R. cascadilla*. However, upon study of the original description and drawing of *R. cascadilla* (57, Fig. 1*a*) and upon taking the measurements into account, one sees that Wigdor mistook the mesostom for the anterior part of the oesophagus and the anterior part of the oesophagus for the posterior portion. There is also a discrepancy between the measurements of the spicule given in Wigdor's text

and those which can be made from his drawing. Wigdor gives the length of the long spicule as 40$\mu$, with the small one being one-fifth as long. The drawing (57, Fig. 1*c*), indicates that the length of the long spicule is 400$\mu$; the value given in the text is obviously erroneous. However, the length of the spicules as given by Wigdor is used by Weller (56) in his discussion of *R. ovifilamenta;* on the other hand, Hsü (29) in his discussion of *R. opiensis* appears to be aware of this error. Gustafson (27) in his discussion of *R. cascadilla* draws attention to these errors.

Another discrepancy in Wigdor's description and illustration of *R. cascadilla* is in the position of the anus. The distance from the posterior extremity is given as 60$\mu$ , whereas according to the scale of the drawing (57, Fig. 1*b*), it is about 130$\mu$. Similarly, the use of this scale enables the length of the anterior portion of the oesophagus to be figured as about 200$\mu$ and to situate the nerve ring at about 115$\mu$ from the anterior end.

Wigdor's description of this species is based upon material collected from *Semotilus a. atromaculatus* and *Notropis cayuga* from Cascadilla Creek, a tributary of Lake Cayuga, New York, U.S.A. The writer is informed by Dr. D. A. Webster of the Fishery Biology Department, Cornell University, that *N. cayuga* was based on what is known to be *Notropis heterolepis heterolepis* and *Notropis bifrenatus*. Since all efforts to trace Wigdor's type material have failed, the writer endeavored to obtain *Rhabdochona* from the type hosts and exact locality of *R. cascadilla*. Thus, in May, 1949, through the kindness of Dr. Webster, *Semotilus a. atromaculatus*, *Notropis cornutus cornutus*, *Rhinicthys a. atratulus*, and *Hyborhynchus notatus* were secured from the type locality of *R. cascadilla*. Only specimens of the first three species of fish were found to harbor a rhabdochonid nematode identified as *R. cascadilla*. However, a few months before (February, 1949) Gustafson submitted for publication a paper (27) in which he redescribes as *R. cascadilla* a form taken from *Semotilus a. atromaculatus* and *Hyborhynchus notatus* from the Embarrass River, Illinois, U.S.A.

The writer had the opportunity to examine some of Gustafson's material and to compare it with forms taken from fish hosts from Cascadilla Creek as well as with other material collected in the Province of Quebec, Canada, and identified as *R. cascadilla*. Measurements of the material taken from *S. a. atromaculatus* are given in Table II. Since the original type material cannot be traced and has in all probability been destroyed, the writer designates specimens from this topotypical series as neotype and neoallotype.

These are small, slender worms with a thin, smooth, unstriated cuticule. The funnel-shaped prostom is provided with 14 teeth which protrude anteriorly and which are well seen in *en face* view (Fig. 2); internally, it has a thick lining which continues posteriorly into the vestibule. At the posterior end of the prostom, at the level of the vestibular opening, the lining is thickened with the internal surface of this thickening protruding forward as sharp points. No teeth were seen at the base of the prostom which is followed by the narrow

## TABLE II

TABLE OF MEASUREMENTS (IN MM.) AND OF RATIOS TO TOTAL LENGTH OF *Rhabdochona cascadilla* WIGDOR, 1918, BASED ON MENSURATION OF 10 MALE AND 7 FEMALE WORMS FROM *Semotilus a. atromaculatus* FROM CASCADILLA CREEK, CAYUGA LAKE, N.Y.

| | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| | Min. | Max. | Average | Ratio | Min. | Max. | Average | Ratio |
| Total length | 3.87 | 5.42 | 4.64 | — | 6.59 | 12.84 | 7.82 | — |
| Oesophagus: length | 1.17 | 1.70 | 1.41 | 1 : 3.3 | 1.60 | 3.09 | 1.92 | 1 : 4 |
| Anterior oesophagus: length | 0.15 | 0.20 | 0.18 | 1 : 26 | 0.20 | 0.31 | 0.23 | 1 : 33 |
| Posterior oesophagus: length | 1.01 | 1.50 | 1.28 | 1 : 3.6 | 1.43 | 2.78 | 1.68 | 1 : 4.6 |
| Buccal cavity: length | 0.014 | 0.016 | 0.015 | — | 0.017 | 0.022 | 0.018 | — |
| Vestibule: length | 0.073 | 0.077 | 0.070 | 1 : 66 | 0.070 | 0.096 | 0.081 | 1 : 98 |
| Excretory pore from anterior extremity | 0.18 | 0.21 | 0.20 | 1 : 23 | 0.20 | 0.27 | 0.23 | 1 : 34 |
| Nerve ring from anterior extremity | 0.12 | 0.14 | 0.13 | 1 : 35 | 0.13 | 0.16 | 0.15 | 1 : 54 |
| Tail length | 0.22 | 0.29 | 0.26 | 1 : 18 | 0.22 | 0.30 | 0.26 | 1 : 30 |
| Short spicule | 0.10 | 0.11 | 0.11 | — | — | — | — | — |
| Long spicule | 0.39 | 0.44 | 0.42 | — | — | — | — | — |
| Length of short spicule to length of long spicule | — | — | — | 1 : 3.9 | — | — | — | — |
| Vulva from anterior extremity | — | — | — | — | 3.32 | 7.11 | 4.20 | 1 : 1.9 or 52% |
| Egg | — | — | — | — | 33$\mu$ × 17$\mu$ | 34$\mu$ × 18$\mu$ | — | — |

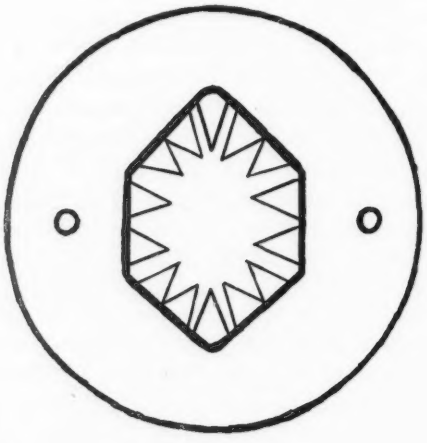

FIG. 2

vestibule opening in the oesophagus which is distinctly divided into a muscular anterior portion and a glandular, much longer posterior portion. The eggs are 33-34$\mu$ by 17–18$\mu$ and are not filamented.

*Female:* Length 6.59 to 12.85 mm.; prostom 17 to 22$\mu$ long; vestibule 70 to 96$\mu$ long; oesophagus, anterior portion is 209 to 312$\mu$ and the posterior portion 1.43 to 2.785 mm. with a total length of 1.609 mm. to 3.097 mm. The nerve ring is placed 134 to 160$\mu$ from the anterior extremity with the excretory pore behind it at 202 to 266$\mu$ from the cephalic end. The vagina is 51 to 54$\mu$ long and the vulva is equatorial in position or a little pre-equatorial. The tail is 215 to 301$\mu$ long.

*Male:* Length 3.877 to 5.423 mm. long; prostom 15$\mu$ long; vestibule 73 to 74$\mu$ long; oesophagus, anterior portion is 154 to 200$\mu$ and the posterior portion 1.017 to 1.503 mm. long with a total length of 1.017 to 1.503 mm. long. The nerve ring is 119 to 144$\mu$ from the anterior extremity and the excretory pore 184 to 212$\mu$ from the anterior end. The right spicule, with a reflected barb at its posterior end, is 102 to 112$\mu$ long and the left one is 385 to 443$\mu$. The tail is 215 to 298$\mu$ long. There are seven or eight pairs of preanal papillae, subventral in position except the third pair from the cloaca which is sublateral; there are six pairs of postanal papillae subventral in position except the second pair, which is sublateral.

Neotype male and neoallotype female: U.S. National Museum Helminthological Collection, No. 47320.

Type host: *Semotilus a. atromaculatus.*

Type locality: Dyer's Pond, Cascadilla Creek, a tributary of Lake Cayuga, N.Y., U.S.A.

Data from the remainder of the topotypical series are as follows: 10 female and 7 male specimens deposited in the Institute of Parasitology collection.

Examination of these data and those given in Table II shows that this description differs from that given by Gustafson (27) only in the number of preanal papillae in the male worm. However, variation in the number of preanal papillae was noted in specimens from *Semotilus a. atromaculatus* collected in the Province of Quebec; in these forms the number of preanal papillae varies between 8 to 12, while in specimens taken from the speckled trout, *Salvelinus fontinalis*, and previously identified as *R. laurentiana* (*vide infra*) the number of preanal papillae range from 9 to 11. From *Catostomus commersonii commersonii*, forms with 14 preanal papillae were seen. Otherwise, if all measurements and ratios are taken into account no clear distinction appears between these forms. Similar variations in the number of papillae were observed by Gnedina (26) in a study of *R. denudatus.*

The size of the worms is quite variable not only in specimens from various hosts but also in specimens from the same host, this variation being more evident in female worms. In his description of *R. cascadilla*, Gustafson (27)

mentions the presence of teeth at the base of the prostom. Examination of some of Gustafson's material as well as specimens collected by the writer, failed to show such teeth.

*The Status of R. laurentiana Lyster, 1940*

This species was first reported by Lyster (42) who described it from *Catostomus commersonii commersonii.* The writer (13) also reported it from *Salvelinus fontinalis* from the Quebec Laurentide Park. Lyster's description was based upon the study of five males and five nonovigerous females. Lyster based the distinction between *R. laurentiana* and *R. cascadilla* on Wigdor's data on the length of the oesophagus; as shown already, this is erroneous. In his description of *R. laurentiana* Lyster noted the absence of a chitinous spine in the male but examination of his material shows this spine to be present. Another distinction made by Lyster was in the number of preanal papillae. However, as shown in the present paper this is subject to variation. Otherwise, *R. laurentiana* is identical with *R. cascadilla.* Therefore, as a result of this study the writer believes that *R. laurentiana* Lyster, 1940 should be regarded as synonymous with *R. cascadilla* Wigdor, 1918.

*Host and Geographical Distribution of Species of Rhabdochona*

In his description of *R. ovifilamenta* Weller (56) mentions that two species, *R. gnedini* and *R. savini,* are attributed to Skrjabin but he does not give any reference pertaining to this subject. Dr. Dollfus (17) informs me that the first species was recorded from *Pseudocaphyrhynchus kaufmanni* and the second one from *Acipenser ruthenus* presumably from Russia in both cases. These two species are mentioned in the list of new species described by Skrjabin in the "Collected papers on Helminthology dedicated by his pupils to K. I. Skrjabin in his Fortieth Year of Scientific, Educational and Administrative Achievement" published in Russia in 1946. However, the writer was unable to see this publication or to find any other reference regarding these two species, except that Layman (36) states that *R. savini* was described by Skrjabin in 1930 and adds a few words concerning its structure. However, Layman does not cite any 1930 paper by Skrjabin in his bibliography.

The following list shows that species of the nematode genus *Rhabdochona* have been reported from most parts of the world, namely from many European countries, parts of Africa, Asia, Australia, New Zealand, and the Americas. At present 33 species are ascribed to this genus. The host list is impressive, species of *Rhabdochona* having been recorded from 100 species of fish, from two undetermined species of fish, and from one crab. Records marked by an asterisk are those of forms identified by the writer, while those marked with two asterisks are forms previously recorded as *species inquirenda* and identified by the writer. The sign "X" preceding the reference number in the list means that the writer could not consult the reference but that they are to be found in the Index Catalogue prepared by Stiles and Hassall (52).

1. *acuminata* (Molin, 1860) Gendre, 1921 (25).

Brasil. *in Barbus* sp. (43, 54, 25), *Brycon falcatus* (54), *Pimelodella laterstriga* (55), *Glanidium neivai* (55).

French Guinea. *in Barbus* sp. (25, 33).

2. *amago* Yamaguti, 1935.

Japan. *in "amago"* (58), *Salvelinus malma* and *Mogurnda obscura* (59).

3. *anguillae* Spaul, 1927.

Spain. *in Anguilla anguilla* (50).

4. *cascadilla* Wigdor, 1918.

U.S.A. (New York State) in *Semotilus a. atromaculatus* and *Notropis heterolepis heterolepis* and *Notropis bifrenatus* as *Notropis cayuga* (Meek) (57), *Notropis cornutus cornutus* and *Rhinicthys a. atratulus* (*).

(Wisconsin) *in Salmo gairdnerii irideus, Salvelinus fontinalis, Cottus b. bairdi* (19, 20), *Rhinicthys c. cataractae, Semotilus a. atromaculatus, Hyborhynchus notatus, Notropis cornutus frontalis, Beleosoma n. nigrum* (19), *Catostomus catostomus* (*), *Hypentilium nigricans* (*).

(Lake Erie) *in Hiodon tergisus, Carpiodes cyprinus, Moxostoma lesueurii, Notropis volucellus, Notropis hudsonius,* and *Notropis whipplii* (1).

(Illinois) in *Semotilus a. atromaculatus* and *Hyborhynchus notatus* (27).

Canada. (Algonquin Park, Ontario) *in Chrosomus eos, Hyborhynchus notatus, Micropterus dolomieu, Leucosomas corporalis* (5), *Semotilus a. atromaculatus, Notropis cornutus frontalis* (5), (1), *Catostomus commersonii commersonii* (*), (5), and *Ameirus nebulosus* (*), (5, 1).

(Quebec) *in Catostomus commersonii commersonii* (42), and *Salvelinus fontinalis* (13) *nec R. laurentiana* Lyster. *Catostomus catostomus* (*), *Semotilus a. atromaculatus* (*), and *Margariscus margarita nachtriebi* (*).

5. *coelorhynchi* Johnston and Mawson, 1945.

New Zealand. (Antarctic Islands) *in Coelorhynchus australis* (32).

6. *conoura* (v. Linstow, 1885) Chitwood, 1933.

Syn: *Filaria conoura* v. Linstow, 1885 (38)

*Spiroptera conoura* (v. Linstow, 1885) v. Linstow, 1909 (41).

*Rhabdochona conoura* (v. Linstow, 1885) Chitwood, 1933 (12).

Europe. (Finland, Germany, and Central Europe) *in Anguilla fluviatilis* (38), *Anguilla vulgaris* (X28, 51, 52, 41, 30), *Aspius* sp. (51), *Aspius rapax* (X28, 51, 52, 41, 30), *Leuciscus* sp. (X28, 52), *Leuciscus grisalgine* (30), *Lota vulgaris* (41, 30), *Perca fluviatilis* (X28, 51, 52, 41, 30), *Squalius lepuscula* (51), *Squalius leuciscus* (41), and *Tinca vulgaris* (41, 30).

7. *cotti* Gustafson, 1949.
   U.S.A. (Washington St.) *in Cottus cognathus* (27).

8. *decaturensis* Gustafson, 1949.
   U.S.A. (Illinois) *in Aplodinotus grunniens* (27).
   (Tenn.) *in Ictalurus punctatus* (**(2) )
   (Wisconsin) *in Hiodon tergisus* (*), *Ictalurus punctatus* (*), and *Moxostoma aureolum* (*).

9. *denudata* (Dujardin, 1845) Railliet, 1916.
   Syn: ? *Fusaria cuneiformis* Zedder, 1800 (46)
   ? *Ascaris cuneiformis* Rud., 1809 (46)
   *Dispharagus denudatus* Dujardin, 1845 (18)
   *Histi ocephalus denudatus* Diesing, 1851 (14)
   *Cucullanus pachystomus* v. Linstow, 1873 (37)
   ? *Dispharagus filiformis* Zschokke, 1884 (X61, 46)
   *Ancyracanthus denudatus* v. Linstow, 1889 (40)
   *Ancyracanthus denudatus* v. Linstow, 1902 (40)
   *Rhabdochona denudatus* (Duj., 1845) Railliet, 1916 (46)
   *Pseudancyracanthus denudatus* Skrjabin, 1923, *in part* (48)
   Europe. France, Denmark, Germany, Central Europe, U.S.S.R., Switzerland. *in Alburnus alburnus* (26), *Alburnus lucidus* (52, 61, 40, X28, 41), *Alcichthys alcicornis* (35), *Barbus fluviatilis* (40, 41), *Bliccopsis abramo-rutiloides*, *Bliccopsis rutiloides* (37), *Cyprinus* sp. (14), *Cyprinus cultratus* (52, X47, 53), *Cyprinus idus* (52, X47, 53), *Cyprinus erythophthalmus* (18, 32, X9). *Gobio fluviatilus* (5, X34, X28, 53, 41), *Gobio vulgaris* (14), *Idus melanotus* (52, 40, X28, 41), *Leuciscus brandti* (35), *Leuciscus cephalus* (26), *Leuciscus idus* (52, X34, 26), *L. rutilus* (31), *Pelecus cultratus* (52, X28, X34, 41), *Phoxinus laevis* (52, 40, X28, 41), *Scardinus erythrophthalmus* (51, 39, 40, 24), *Squalius cephalus* (52, 61, 62, X28, 41), *Squalius leucosus* (41).

   NOTE: A number of these species is mentioned by Skrjabin (48) but he does not say whether or not these actually occur in Russia.

10. *denudata-filamentosa* Bychowsky (Pawlowsky), 1936.
    U.S.S.R. *in Schizothorax pseudoksaiensi* (9).

11. *elegans* Travassos, Artigas, and Pereira, 1928.
    Brasil. *in Tetragonopterus* sp. (lambori) (54).

12. *fujiii* (Fujita, 1921) Fujita, 1927.
    Japan. *in Onchorhynchus nerka*, *Salvelinus kundscha* (22).

13. *fortunatowi* Dinnik, 1933.
    U.S.S.R. *in Varicorhinus copoetus sevangi* (15).

14. *gambiana* Gendre, 1921.
French Guinea. *in* undetermined species of fish (25, 33).

15. *girellae* Yamaguti, 1935.
Japan. *in Girella punctata* (58).

16. *gnedini* Skrjabin. (*supra cit.*).
U.S.S.R. *in Pseudocaphyrhynchus kaufmani.*

17. *gymnocranii* Yamaguti, 1935.
Japan. *in Gymnocranius griseus* (58).

18. *helliki* (Sramek, 1901) Chitwood, 1933 (10).
Now part of modern Czechoslovakia *in Barbus fluviatilis* (51, 52, X28), *Squalius dobula* (51), *Squalius cephalus* (51, ×28).

19. *jaenschi* Johnston and Mawson, 1940.
Australia. *in Pseudophritis urvillei* (31).

20. *kidderi* Pearse, 1936.
Yucatan. *in Rhamdia guatemalensis depressa* (45), *Rhamdia guatemalensis decolor* (45, 12), *Rhamdia strygea* (45), and *Typhlias pearsi* (12).

21. *macrolaima* Gendre, 1921.
French Guinea. *in* undetermined species of fish (25, 33).

22. *milleri*, nov. sp.
Canada (Quebec). *in Moxostomus aureolum* (*).

23. *onchorhynchi* Fujita, 1940.
Japan. *in Onchorhynchus keta* (24).

24. *opienensis* Hsü, 1933.
China. *in Oreinus sinensis* (29).

25. *ovifilamenta* Weller, 1938.
U.S.A. (Michigan State) *in Perca flavescens* (56).

26. *paski* Baylis, 1928.
Africa (Lake Tanganyika). *in* the "Tanganyika salmon"? *Alestes macrophthalmus*, a species of siluroid fish and in one undetermined species of fish (6).

27. *pellucida* Gustafson, 1949.
U.S.A. (Washington St.) *in Pteichocheilus oregonensis* (27).

28. *salvelini* Fujita, 1927.
Japan. *in Salvelinus fluvius* (21, 23).

29. *savini* Skrjabin, 1930. (*supra cit.*).
U.S.S.R. *in Acipenser ruthenus.*

30. *tridentigeris* Yamaguti, 1940.
Japan. *in Tridentiger obscurus obscurus* (59).

31. *turkestanica* (Skrjabin, 1917)
U.S.S.R. *in Schizothorax intermedius* (48).

32. *uca* Pearse, 1932.
India. *in Uca manii* (44).

33. *zacconis* Yamaguti, 1935.
Japan. *in Zacco platypus* (58), and *Liobagrus reini* (57).

34. sp.
Russia. *in Barbus brachycephalus* (16).
Canada. (Algonquin Park, Ontario) *in Perca flavescens, Lepomis gibbosus, Coregonus clupeaformis* (3, 5), *Pimephales promelas* (5).
U.S.A. (Lake Erie) *in Lepibema chrysops* (1), *Helioperca incisor*, and *Ambloplites rupestris* (1).
(Wisconsin St.) *Rhinicthys atratulus meleagridis* (3).

## Acknowledgments

The writer wishes to express his gratitude to Dr. R. V. Bangham of Wooster College, Ohio, and to Dr. J. H. Fischthal of Syracuse University, for the loan of some of the material used in this study and for the information they so willingly forwarded. I am also indebted to Prof. Dwight A. Webster of Cornell University, Ithaca, N.Y., for his cooperation in collecting material from Cayuga Lake. I wish also to express my gratitude to Dr. E. W. Price of the U.S.A. Bureau of Animal Industry and to Dr. R. Ph. Dollfus of the Museum d'Histoire Naturelle de Paris, for their aid in securing much needed references.

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# THE POLYHEDRAL DISEASE OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.) (LEPIDOPTERA: TORTRICIDAE)[1]

By G. H. Bergold[2]

## Abstract

In investigations of the polyhedral disease of the spruce budworm, the polyhedral bodies have been purified and the isolated virus rods described. Quantitative infection tests indicate a high resistance or immunity of the insects against the virus. Some remarks are made regarding epizootiology.

## Introduction

The spruce budworm, *Choristoneura fumiferana* (Clem.), is one of the most destructive native insects in the balsam and spruce forests in North America, and the heavy losses of timber have prompted intensive investigations of diseases that might be used in control. Recent advances in the field of virus diseases of insects (3, 8, 4, 5, 15) encouraged the work on this problem. Short notes on virus diseases of this insect have been given previously (12, 13, 6, 11).

It was found in Europe in 1948 that *Cacoecia murinana* (Hb.) was heavily infected with a capsule virus disease (5) [= granulosis, *Bergoldia calypta* Steinhaus (15, 16)]. *Choristoneura fumiferana* is very closely related to *Cacoecia murinana*. Therefore, it was attempted to infect the Canadian budworm with the European capsule virus. Purified capsules were fed, and liberated free virus rods were injected. Several experiments proved that the European capsule virus was highly specific and was not lethal to *C. fumiferana* in direct transfer. The capsule virus provoked, however, the manifestation of a latent polyhedral disease. In the meantime, this polyhedral disease was also found in dead larvae of the spruce budworm from various localities in Ontario. In comparison with the polyhedral disease, there is little known about the capsule disease of insects (5, 17, 18). It was decided, therefore, to investigate first the polyhedral disease of *Choristoneura fumiferana*.

## Materials and Methods

### Rearing of Insects

The rearing stock of budworms originated from a field collection of several hundred pupae from one locality in Ontario (Island Falls, near Cochrane), in 1948. They have been reared for three generations in the laboratory, according to the following technique:

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After emergence from the egg mass the larvae were left in glass jelly jars (8 oz.) at room temperature until they had spun hibernacula in cheesecloth (about one to four weeks). After spinning they were kept at a temperature of about 6° C. for one week and then at about 1° C. for four to six months. The larvae became active after being exposed to about 22° C. and about 60 to 80% relative humidity. They were fed in the summer months with fresh and in the winter months with deep-frozen young buds of balsam fir, *Abies balsamea* (L.) Mill., with equal success. Larvae were reared in small ground Petri dishes (60 × 15 mm.), about 20 to a dish, until the early part of the fourth instar, usually without change of food. Fresh food was supplied to the later stages. The larvae pupated in about four weeks. The moths mated in glass jars (16 oz.) and the females laid about 100 to 200 eggs each. From January, 1949, to January, 1950, 17,056 larvae were brought out of storage; 9816 (57.5%) developed successfully and were used for experiments and restocking.

## Purification of the Polyhedral Bodies and Isolation of the Virus

Generally it is found that the polyhedral disease of the spruce budworm takes the course characteristic of the classic example of a polyhedral virus, that of the silkworm, *Bombyx mori* (L.) (2). The same methods for isolation and characterization of the infectious virus particles can be applied (3).

Smears of larvae dead from the polyhedral disease show in the dark field at 1500× enormous amounts of the characteristic polyhedral bodies. These dead larvae were left suspended in water for several days, while the polyhedral bodies settled in a white layer at the bottom of the Erlenmeyer flask. The suspension was filtered through fine nylon tissue and was fed to larvae by direct application and by spraying the food. After three passages there were enough dead larvae to isolate the polyhedral bodies. The purification is rather easy by centrifugation of the water suspension at different speeds in a Sorvall centrifuge, type SS-1*a*, using lucite beakers. The polyhedral bodies were finally obtained as a white powder. The average yield is approximately 0.25 mgm. per full-grown larva. About 1 gm. of pure polyhedral bodies has been obtained so far.

The infectious virus particles are occluded in the polyhedral bodies. They can be liberated by dissolving the polyhedral bodies in weak alkaline solution. The strength of the alkaline solution varies with the quantity of polyhedral bodies; 0.007 *M* to 0.03 *M* sodium carbonate + 0.05 *M* sodium chloride may be used to dissolve 1 to 5 mgm. per ml. It was found that a few of the polyhedral bodies do not dissolve completely. After about three hours the incompletely dissolved polyhedral bodies and impurities may be sedimented at 6000 r.p.m. for five minutes. The slightly turbid solution of polyhedral protein and virus particles is then spun for one hour at about 11,500 r.p.m. The clear supernatant, representing the polyhedral protein, is poured off. The small amount of bluish-white sediment of virus particles is suspended in distilled water and sedimented again for one hour at 11,500 r.p.m. The supernatant

is discarded and the virus suspended in one-seventh of the starting volume in 0.0001 *M* sodium carbonate, in which it suspends better than in distilled water.

### Testing

Dark field examinations with the light microscope as well as infecting experiments proved that the rearing stock is latently infected with a microsporidium and with two virus diseases: a polyhedral and a capsule disease. Normally only small numbers of the larvae showed typical symptoms of the virus diseases, and died.

The contamination of the test insects with three diseases complicated the investigations enormously. The use of large numbers of test insects, quantitative testing, and statistical calculations (use of probability net) were necessary (3, 14).

For oral infection, small droplets of a test solution, consisting of a polyhedral suspension, were applied once or more often to the mouth parts of the larvae. Frequently, the food also was sprayed with the test solution.

For the quantitative tests, one-microliter quantities of a test solution consisting of purified virus particles were injected into the early fourth-instar larvae with a microsyringe with the aid of a dissecting microscope (1). The injection of the small larvae (about 3–5 mm. in length) is facilitated when the larvae are anesthetized with ether. The glass capillary of the microsyringe was inserted in the tip of a proleg. In this way, the ring muscle closes the insertion hole, and no bleeding takes place. The virus concentration was defined by micro-Kjeldahl determination of nitrogen and multiplying the amount of nitrogen by 6.25. The dilutions were made with sterile distilled water. Usually 60 to 90 insects were used for one test. They were divided into three groups of 20 to 30 insects. In the tests, three orders of concentration were covered, by applying to each succeeding group a solution 10 times as dilute as the preceding one.

Larvae were kept singly in small Petri dishes. Extreme precautions to maintain sterility were observed. Every dead larva was examined individually in the dark field of a Leitz Ortholux microscope (oil dark field condenser N.A. 1.20, oil immersion 95 ×). Sometimes the diagnosis was checked in the electron microscope, an R.C.A. Electron Microscope, Type EMU, being used. No shadow-casting was carried out, but the virus preparations were fixed by exposure to vapors of a 2% osmium tetroxide solution.

Only the dead larvae that showed the typical symptoms of the disease and specimens that developed into adults were used for the calculations of the $LD_{50}$. Larvae that died from other causes were rejected.

## Results

Fig. 1 is a characteristic picture of the polyhedral bodies of the spruce budworm in suspension. Their size varies from about 1 to 2.5 $\mu$. Their shape is rather irregular, but cubical and tetrahedral forms are rather frequent.

Fig. 2 shows polyhedral bodies in dissolution. Virus bundles or developing stages and single virus rods seem to occur at the same time within one polyhedral body. The small, spherical particles with diameters of about 10–20 mμ seem to represent the elements that form the polyhedral membrane. They may be aggregation products of polyhedral protein molecules.

Only about 1.5% by weight of the polyhedral bodies consists of virus particles, which is about one-third the amount of virus in silkworm polyhedral bodies.

Electron micrographs of such virus suspensions show rods that are characteristic of insect viruses (Fig. 3). The average length* of 273 virus rods was 260 mμ and the average diameter about 28 mμ. The developing stages of the rods have been shown and discussed in a previous paper (7).

Preliminary chemical analyses** of the nitrogen–phosphorus ratio (9) of the polyhedral bodies, the polyhedral protein, and the virus particles are given in Table I. The phosphorus content of the virus is high and that of the polyhedral protein is low, as for polyhedral material from other insects (3). All the phosphorus of the virus is bound as thymonucleic acid phosphorus (10).

TABLE I

THE NITROGEN–PHOSPHORUS RATIO OF THE POLYHEDRAL BODIES, THE POLYHEDRAL PROTEIN, AND THE VIRUS OF THE POLYHEDRAL DISEASE OF THE SPRUCE BUDWORM

| | Polyhedral bodies | Polyhedral protein without virus | Polyhedral protein dialyzed | Virus |
|---|---|---|---|---|
| N/P | 54 | 68 | 200 | 16 |

## THE INFECTIVITY OF THE POLYHEDRAL BODIES AND OF THE FREE VIRUS PARTICLES

The infectivity of the polyhedral bodies was investigated by applying heavy doses per os. Table II indicates that about six % of the larvae are resistant and develop into adults. It was found that larvae infected in

TABLE II

MORTALITY AND SURVIVAL OF SPRUCE BUDWORM LARVAE AFTER ORAL INFECTION WITH POLYHEDRAL BODIES

| Total | Died of polyhedral disease | | | | Developed to adults | |
|---|---|---|---|---|---|---|
| | As larvae | | As pupae | | | |
| | No. | % | No. | % | No. | % |
| 2224 | 2027 | 91 | 49 | 2 | 148 | 6 |

* *These dimensions have still to be checked with shadowed preparations.*
** *These analyses will be checked if more material is available.*

PLATE I

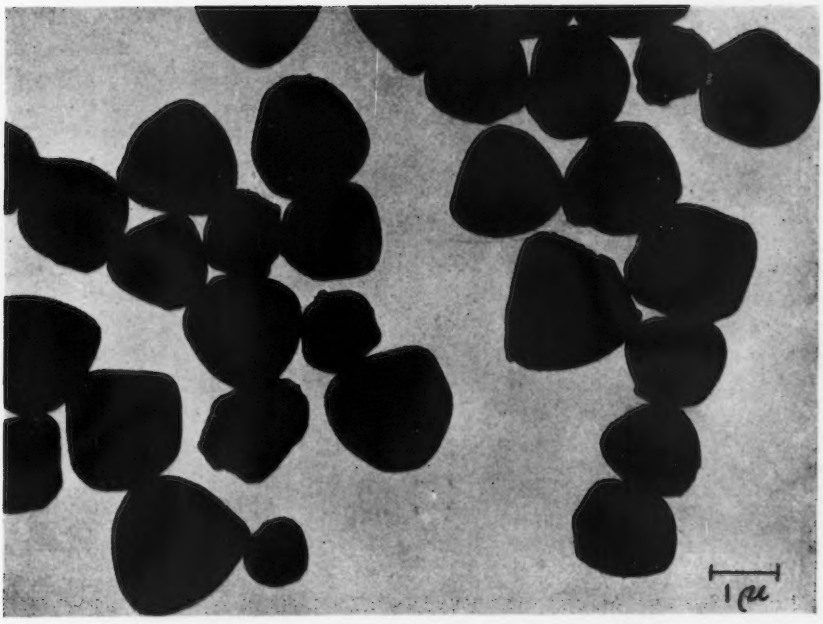


FIG. 1. Purified polyhedral bodies from the spruce budworm, *Choristoneura fumiferana* (Clem.). Electron micrograph magnification 10,000×.

PLATE II

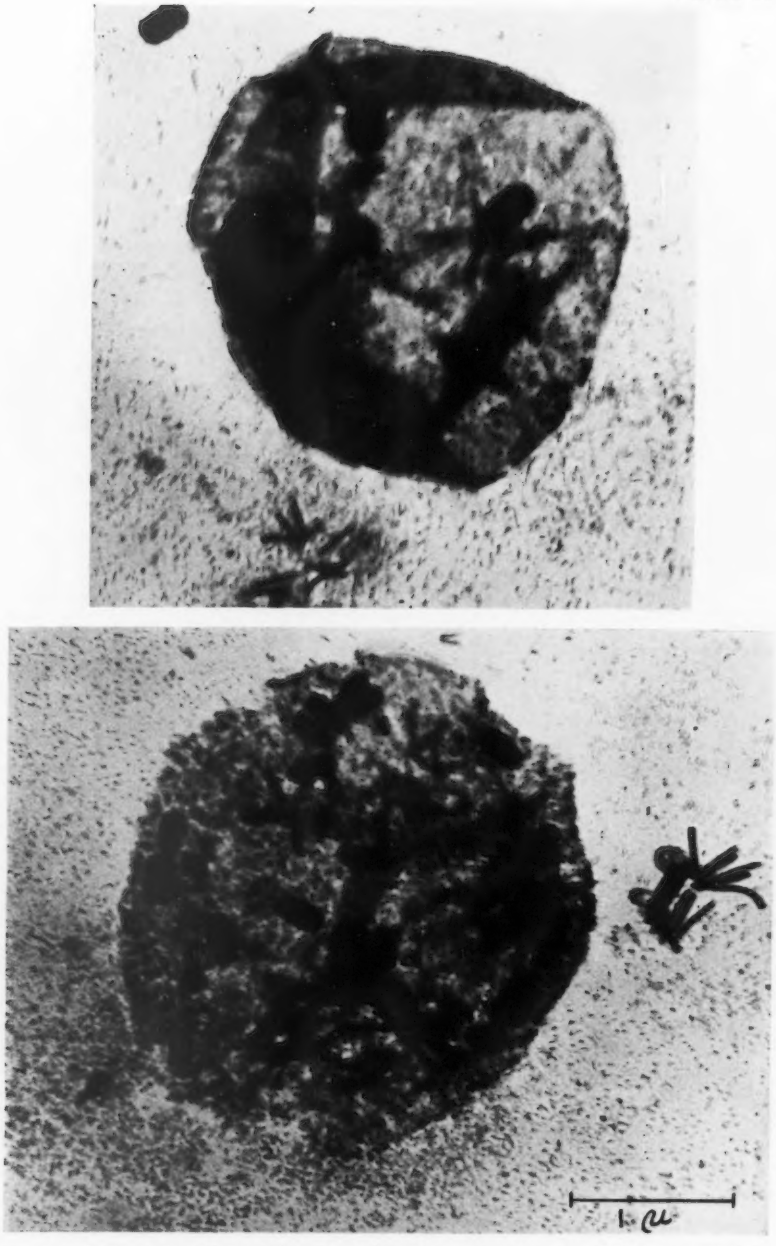


FIG. 2. Polyhedral bodies of the spruce budworm, *Choristoneura fumiferana* (Clem.), in dissolution. Virus rods and bundles or developing stages can be seen inside the polyhedral bodies. The small, almost spherical particles about 10 to 20 mμ in diameter seem to be the elements that form the polyhedral membrane. Electron micrograph magnification 25,000×.

PLATE III

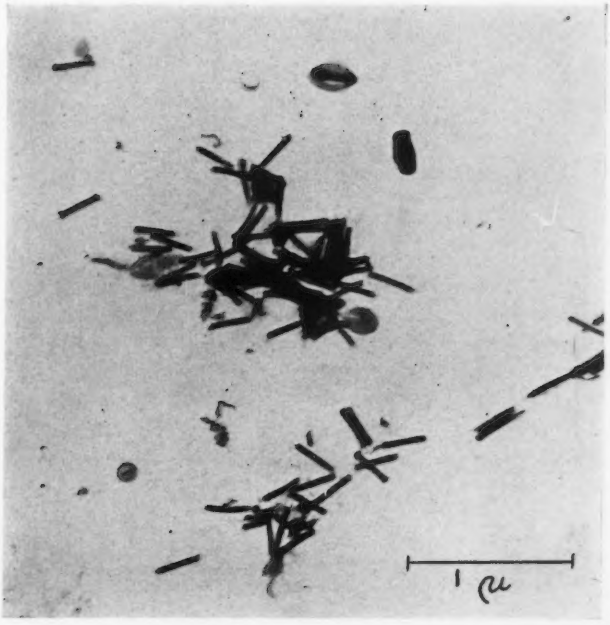


FIG. 3. Mature rods and developing stages of the virus particles of the spruce budworm, *Choristoneura fumiferana* (Clem.). Electron micrograph magnification 25,000×.

the last stage, about 10 days before pupation, frequently pupated and developed into adults. This is characteristic of polyhedral diseases of other lepidopterous insects and is accounted for by the fact that the development of the disease is almost stopped during pupation. The average period from oral infection to death of larvae kept at about 22° C. is approximately 10 days (Fig. 4).

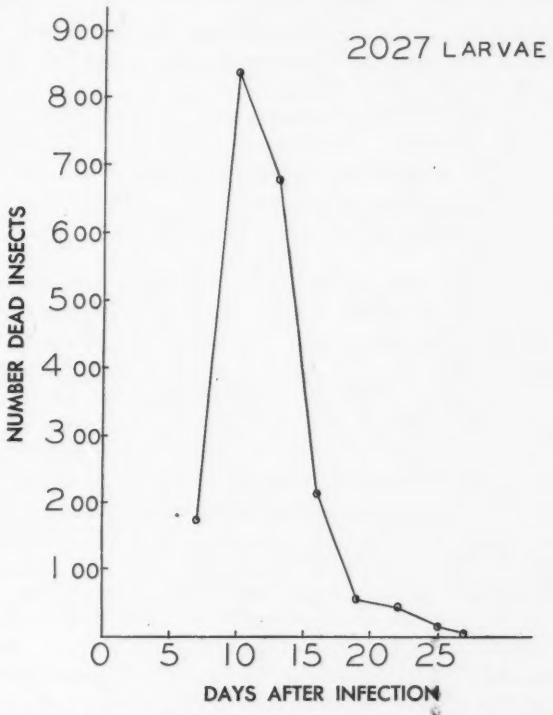


FIG. 4. The period from oral infection with polyhedral bodies to the death of the spruce budworm, *Choristoneura fumiferana* (Clem.).

The quantitative injection tests indicate that about $1.5–3.6 \times 10^{-9}$ gm. of virus particles are necessary for the $LD_{50}$. Table III shows the results of

TABLE III

RESULTS OF A QUANTITATIVE INJECTION WITH THE SPRUCE BUDWORM

| Expt. No. | No. of larvae | Concentration of virus suspension VCf $3/6_2$ in gm. | Larvae dead from polyhedral disease | | Larvae developed to adults | $LD_{50}$ gm. |
|---|---|---|---|---|---|---|
| | | | No. | % | | |
| 516 | 19 | $4 \times 10^{-10}$ | 7 | 37 | 12 | |
| 517 | 25 | $4 \times 10^{-9}$ | 13 | 52 | 12 | $1.5 \times 10^{-9}$ |
| 518 | 23 | $4 \times 10^{-8}$ | 21 | 91 | 2 | |

one such test. This virulence did not change after one further passage. The amount of about $1.5 \times 10^{-9}$ gm. is unusually high in comparison with $4 \times 10^{-13}$ gm. of virus in the case of the silkworm (4). That means that about 5000 times the $LD_{50}$ of the silkworm has to be applied to the budworm, notwithstanding the fact that the silkworm is about 10 times as large.

## Discussion

The above results of investigations on the polyhedral disease of the spruce budworm do not add much to the fundamental knowledge of polyhedral diseases, but they are important for the epizootiology.

Characteristic of an efficient virus disease is a rather sharp decrease from a very dense population of insects to the normal number of individuals within one or two generations as observed in *Porthetria dispar* (L.), *Lymantria monacha* (L.), and *Diprion* (*Gilpinia*) *hercyniae* (Htg.). This situation seems not to occur in spruce budworm infestations. Why does the polyhedral disease not become an epizootic?

Three factors chiefly determine an epizootic: "(1) the infectious agent with its variable virulence and infectivity, (2) the susceptibility or resistance of the individuals that compose the population at risk, and (3) an efficient means of transmission" (15, p. 179). Concerning factor (1): the polyhedral virus occurs in nature in the spruce budworm infestations and has a low virulence in laboratory experiments. At present it cannot be decided whether the low mortality of the budworm in the quantitative injection experiments is due to a low virulence of the virus or whether, under factor (2), the budworm populations are highly resistant or/and immune because of previous sublethal infection with the enzootic polyhedral virus. The latter is much more probable, since the budworm is a native insect. That a certain percentage of budworms orally infected with heavy doses develop into adults also points towards immunity. Under (3) two points may greatly influence transmission and dissemination in nature. The feeding period of the budworm is rather short. On the other hand, the time from infection to death (10 days) is rather long. Healthy individuals have, therefore, not much chance to infect themselves—early enough before their own pupation—by contact with the dead ones. At present it is not known whether the polyhedral disease is carried by the eggs into the next generation. If it is, the small, second-instar larvae will die inside their webs and healthy larvae will have no chance to become infected by contact with them.

All these points together may have some influence on the efficiency and distribution of the polyhedral disease of the spruce budworm.

## Acknowledgments

The writer wishes to express his appreciation to the following technicians of the Laboratory of Insect Pathology: J. M. Burk and Miss I. Wingfelder for rearing the larvae; Miss B. Ferguson for the inoculation of the larvae and the diagnostic examinations; and Miss S. Bushila for rearing the infected larvae throughout the experiment.

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# POPULATION CYCLES AND COLOR PHASE GENETICS OF THE COLORED FOX IN QUEBEC[1]

By L. Butler[2]

## Abstract

The pelt collection figures for colored fox in the province of Quebec were examined. The figures for the central and southern part of the province show a typical nine-year cycle. In the northern sections the data show that until 1930 there was a nine-year cycle in colored fox coexisting with a four-year cycle in white fox. After 1930 the four-year and nine-year cycles exist simultaneously with the four-year gradually dominating the scene. In the Upper James Bay region the typical nine-year cycle shows a supplementary peak corresponding with the four-year peak observed in the regions to the north. The coat color phase ratios cannot be explained by monohybrid equilibrium but they are consistent with the view that the population consists of isolates. A partial breakdown of isolate barriers would account for the ratios observed without the necessity of the large unexplained gene frequency changes which occur if panmixia is postulated. Migration causes the breakdown of isolate barriers and this accounts for the sudden shift in gene frequency and explains the long term trends which have resulted in a lower percentage of the silver phase. The degree of isolation changes with the population pressures. The cause of the cycles appears to be resident in the respective area in which the animal breeds. Both the cycle and the color phase data indicate that northward migrations have taken place.

## Introduction

The fur returns and most probably the populations of many Canadian animals (e.g. fisher, lynx, marten, mink, muskrat) show a definite cycle or periodicity. The underlying cause for such periodicity is not known although various theories have been proposed attributing the cause to factors such as climate, sun spot cycles, predation, vitamin deficiencies, and excesses. A few of the facts concerning these periodicities are:

1. The length of cycle for animals which inhabit the area south of the timber line is 9 to 10 years.
2. The length of cycle for animals north of the tree line or in the Arctic tundra is four years.
3. Any animal which occurs in both Arctic and nonarctic regions shows the characteristic cycle of the region in which it is living.

The northern tree line is customarily taken as the line of demarcation between the two lengths of cycle but nothing has been published to show whether there is a clean break at this point or a band of transition between the two cycle lengths. Such knowledge would contribute substantially to our knowledge of cycles. If the line is clean-cut then the causal factor or factors must be resident in the respective areas. If there is a transition

[1] *Manuscript received September 26, 1950.*

*Contribution from Department of Zoology, University of Toronto, Toronto, Ont. The work was partially conducted under a grant from the National Research Council. The data were furnished by the Fur Trade Department of the Hudson's Bay Company.*

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region then we have two interpretations; it may be the result of migration from one region into another or it may be a gradual change-over in the causal factor or factors.

In order to study these cycles we need first of all fur returns for some animal which occurs in both regions. The Hudson's Bay Company pelt returns for colored fox taken in Quebec furnish such data.

Fig. 1 is a map of the Province of Quebec which shows the main collecting posts of the Hudson's Bay Company. The whole of this collecting area lies on the Canadian shield which is not good fox habitat like the savanna country

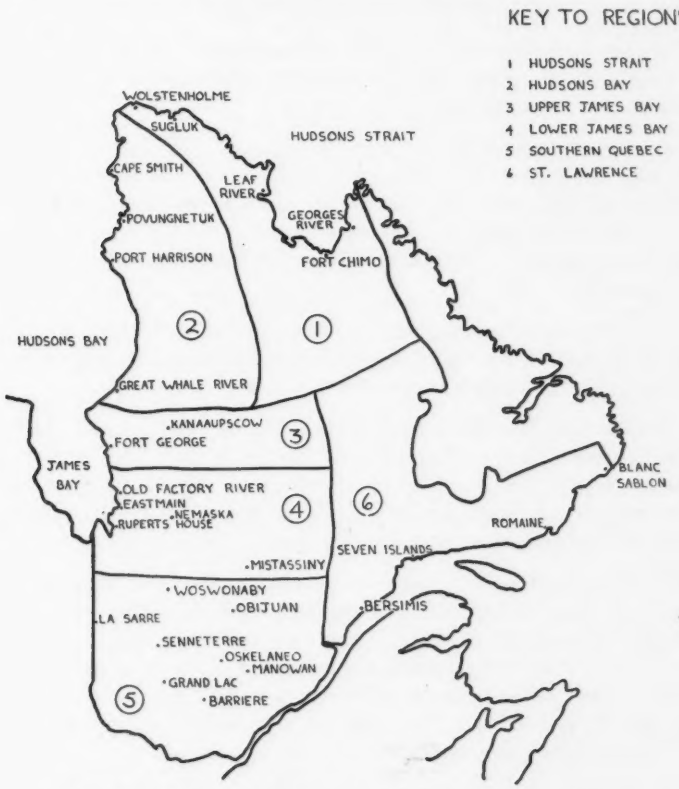


FIG. 1. Map of Quebec showing collecting posts and regions.

of Alberta and Saskatchewan. The yearly collections at any one post are therefore small even in peak years so it is necessary to group the returns of several posts together for the purpose of analysis. The grouping employed is also shown on Fig. 1. These groups consist of fairly homogeneous regions with the following characteristics:

The Hudson Strait region is mostly tundra with some stunted scrub trees in the river valleys of the southern interior part of the region. It is north of the recognized timber line. Its fur crop consists chiefly of white and colored fox with a few mink, muskrats, and ermine. The chief food for the predators consists of lemming (*Dicrostonyx hudsonius*) and mice (*Microtus pennsylvanicus, M. enixus, Phenacomys ungava, Clethrionomys gapperi ungava*). These rodents in the years of their periodic abundance provide rich fare for the fox. The snowshoe rabbit (*Lepus americanus*) is not native to the region but there is a sparse population of the arctic hare (*L. arcticus labradorius*). The collecting posts in this section are: Fort Chimo, Georges River, Payne Bay, Leaf River, Wolstenholme, and Sugluk.

The Hudson Bay Region has similar characteristics and fauna to those of the previous section. The collecting posts are: Povungnetuk, Port Harrison, Cape Smith, and Great Whale River.

The Upper James Bay Region is south of the timber line and produces a wide variety of fur including beaver, marten, mink, and otter. White fox occurs only rarely in this region, consisting of migrants taken on the coast or nearby islands. The lemming is replaced by mice and the snowshoe rabbit is native to the region. The collecting posts in this region are: Fort George and Kanaaupscow.

The Lower James Bay Region has similar characteristics and fauna to those found in the last mentioned region. The collecting posts are: Eastmain, Old Factory River, Rupert's House, Nemaska, and Mistassiny.

Southern Quebec Section contains some cleared land around the mining towns and presents a more favorable habitat for foxes. This section unlike the previous four has no barrier to migration from the west. The collecting posts in this region are: La Sarre, Senneterre, Oskelaneo, Grand Lac, Woswonaby, Obijuan, Manowan, and Barriere.

The St. Lawrence Section consists of posts located on the north shore of the St. Lawrence River and Gulf. The trappers from these posts have trapping grounds as far north as Mistassiny and the Labrador boundary. Because of the extensive trapping grounds the collection from these posts is not as homogeneous and is likely to reflect changes in both northern and southern populations of fur-bearers. The posts in this section are: Seven Islands, Blanc Sablon, Moisie, and Bersimis.

A more detailed account of the ecology, geography, and fur history of the Ungava and Labrador peninsula together with pertinent references is given in Elton (7).

## Analysis of the Population Cycles

The easiest method of finding the length of the cycle is to plot the data and read off the year of the peak population from these graphs (Fig. 2). If we

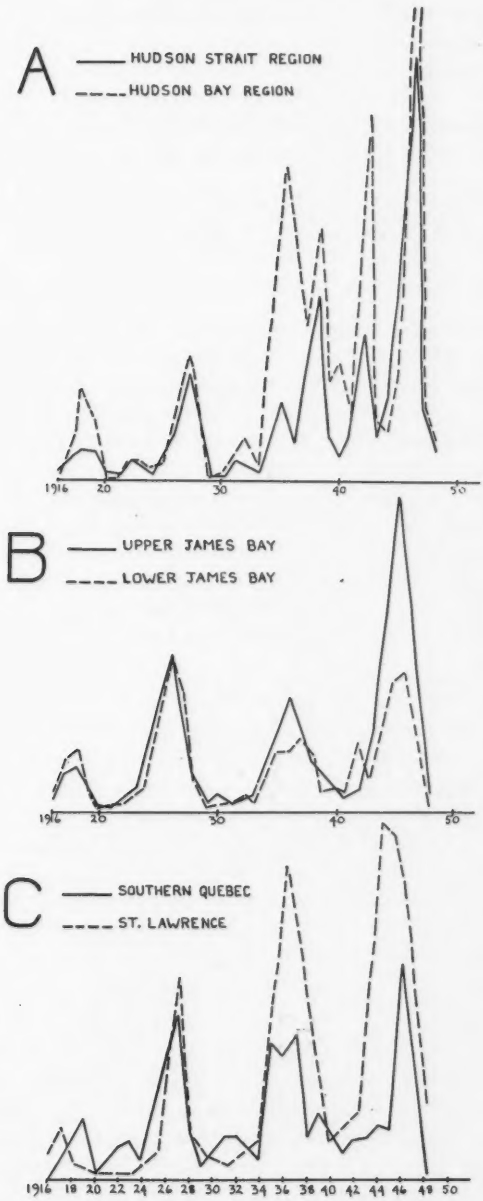


FIG. 2. Yearly pelt collections of colored fox by regions. A. ———— Hudson Strait Region. - - - - - Hudson Bay Region. B. ———— Upper James Bay. - - - - - Lower James Bay. C. ———— Southern Quebec. - - - - - St. Lawrence.

tabulate the peak years listing the regions from south to north we get the following results:

| Region | Peak years | | | | | | |
|---|---|---|---|---|---|---|---|
| St. Lawrence | 1919 | 1927 | — | — | 1936–37 | — | 1946 |
| South Quebec | 1917 | 1927 | — | — | 1936 | — | 1944 |
| James Bay | 1918 | 1926 | — | — | 1936 | — | 1945 |
| Upper James Bay | 1918 | 1926 | — | — | 1937 | 1942 | 1946 |
| Hudson Bay | 1918 | 1927 | 1931 | 1935 | 1938 | 1942 | 1946 |
| Hudson Strait | 1918 | 1927 | 1932 | 1935 | 1938 | 1942 | 1946 |

The first four sections show a typical nine-year cycle with peaks occurring at approximately the same time in each section. The last two sections show a four-year cycle with the 1918, 1927, and 1946 peaks coming at the same time as the nine-year peaks farther south while the 1936-37 peak was straddled by the 1935 and 1938 peaks. The amplitude of these peaks shows in Fig. 2 where the data for each of the six sections is plotted on the same abcissa and scale for comparative purposes. The two southern sections (Fig. 2*a*) are very similar except that the St. Lawrence section has some indication of small intermediate peaks and a split peak in the 1936-37 period. These differences are expected because of the habit of trappers from such St. Lawrence posts as Seven Islands going far north to obtain part of their catch. Thus peak collections in the north would be reflected to a limited extent in the collections from this region.

The next two lines for the James Bay region (Fig. 2*b*) are quite similar except that the northern one has a smaller peak in both 1937 and 1946 and has an intermediate peak in 1942. Upper James Bay section is clearly a nine-year type of cycle until 1942 at which time it seems to have changed over to the four-year type exhibited by the two Northern sections. The data for Hudson Bay and Strait Sections (Fig. 2*c*) show a fairly well defined nine-year cycle up until 1935 although there are slight indications in the two troughs of supplementary peaks. After 1935 the peaks occur at approximately four-year intervals. The 1946 peak is the largest of the lot being over twice as high as any preceding peak.

Generalizing from the data in Fig. 2 we find that the province of Quebec south of Fort George has a typical nine-year cycle. In the Fort George region the 1942 peak is a supplementary one which occurs at the same time as a peak in the arctic four-year cycle. The Hudson Bay and Strait regions show a greatly increased collection from 1935 to date. The fluctuations do not have the cyclic regularity of the other regions but appear to be mixtures of a nine-year and four-year cycle. To get further information on these last two regions we can use more refined methods of analysis.

A more exact method of treating these cyclic data is by means of moving averages of various lengths. If the data analyzed by the four-year moving

average has a perfect four-year periodicity then a plot of this average will be a straight line. The line will be horizontal if no upward or downward trend is present in the data. If the data are mixtures of two cycles the four-year moving average will remove the four-year cycles and allow the other cycle to be seen. In order to make the periodicity more apparent the trend can be calculated and the moving averages plotted around the trend line.

The Hudson Bay area four- and nine-year moving averages are plotted in Fig. 3. The nine-year average is almost a straight line from the beginning

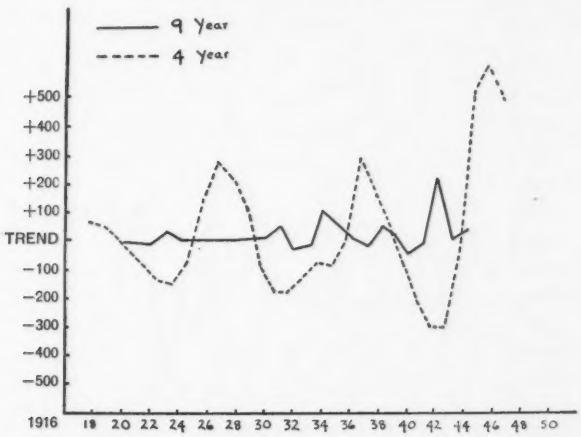


FIG. 3. The moving averages of pelt collections for Hudson Bay regions.

until 1930. After this it shows peaks in 1934, 1938, and 1942 which are the four-year peaks picked out of the data by inspection. The four-year moving average shows a nine-year cycle with peaks in 1927, 1937, and 1946. With the limited data at our disposal it is impossible to try the combination of the four- and nine-year to determine whether both cycles are eliminated.

In Fig. 4 the data for the Hudson Strait area are plotted around the trend line. The plot of the four-year moving average shows a typical nine-year cycle with the exception of the points for 1941 and 1942 which are higher than they should be. The peak years for these nine-year cycles are 1918, 1927, 1937, and 1945-46 which are identical with those in the Hudson Bay section plotted in Fig. 3. The four-year moving average has eliminated the 1932, 1935, 1938, and 1942 peaks thus allowing the underlying nine-year peaks to show up. This is especially true in the case of the 1937 peak. In the original data plotted in Fig. 2*c* the 1937 peak does not show in either the data for the Bay or Strait because of the masking effect of adjacent peaks occurring in 1935 and 1938. Thus the use of the four-year moving average reveals that all four of the nine-year cycle peaks occurred throughout the entire area.

The nine-year moving average from the beginning until 1930 is almost a straight line indicating that only the nine-year cycle is operating in this period. After 1930 the elimination of the nine-year cycle reveals a clearly defined four-year one with peaks in 1931-32, 1934, 1938, and 1942. It should be noted that the 1935 peak was an artifact introduced because of the rising phase of the nine-year cycle which was to peak in 1937 and the falling phase of the four-year cycle gave a combined collection which was higher than the previous peak of four-year cycle. The use of the nine-year average shows that the true year of the peak was 1934 not 1935.

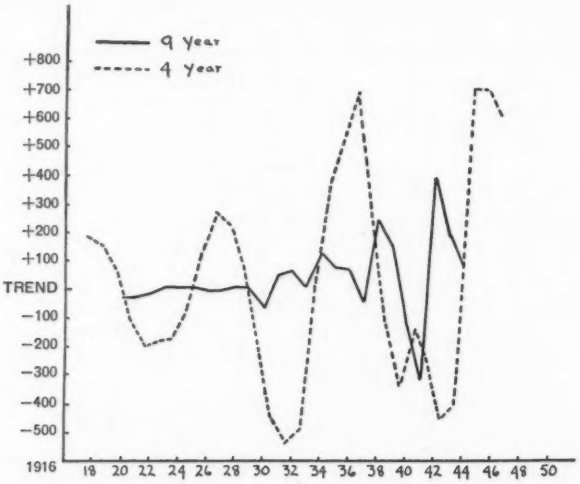


FIG. 4. The moving averages of pelt collections for Hudson Strait region.

We would interpret this as meaning that until the early 1930's only the nine-year cycle was operating in these two northern sections and that at this time the four-year cycle began to dominate the population.

Does this four-year cycle which appears in the northern returns in recent years correspond to the four-year cycle shown by the resident population? We can answer this by comparing the colored fox and white fox returns for the same region. In Fig. 5 the white fox returns for the Hudson Strait region are plotted. From this and Fig. 2*c* we see that the peaks occur in the following years:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| White fox | 1918 | 1922 | 1927 | 1931 | 1934 | 1938 | 1941-42 | 1946 |
| Colored | 1918 | | 1927 | 1931-32 | 1934 | 1938 | 1942 | 1946 |

Thus the colored and white correspond, both peak at the same time. The amplitude of the peaks bears no resemblance to one another, the 1922 white fox peak being the largest and the 1946 peak being the largest for the colored fox.

In the face of this evidence the answer to the question of whether there is a definite boundary between the four-year and nine-year cycle is no. In the 1920's the northern regions had a four-year cycle of arctic fox coexisting with a nine-year cycle of colored fox. In later years the northern sections show that both the nine- and four-year are existing simultaneously in the same population. Since the division between the two types of cycle is not clear-cut one is faced with two alternatives. 1. Does the underlying factor which causes these cyles spill over from one area to the other? 2. Does the causal factor have a definite division but the pressure of southern peak populations cause migration across the boundary line so that both cycles are felt in this northern region?

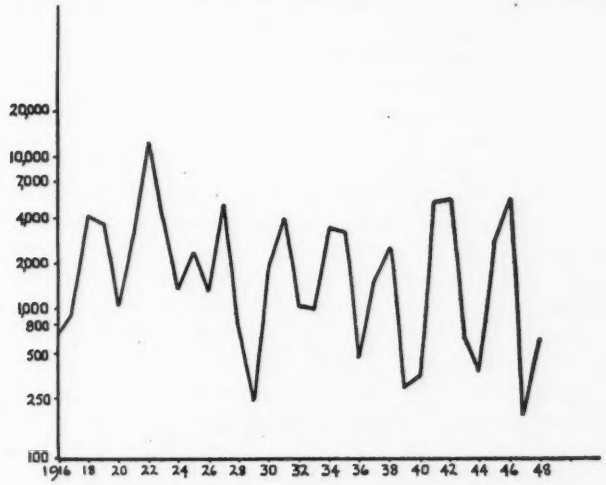


FIG. 5. White fox collections in Hudson Strait region.

The first alternative does not seem plausible or one would expect the amplitude of the white fox cycle to reflect the simultaneous occurrence of a four- and nine-year cycle peak. It does not. The second alternative or migration theory is supported by the evidence so far presented. The white fox cycle is not influenced by the nine-year cycle because there are no breeding grounds in the region where the nine-year cycle operates and thus no migrant population to come in. The red fox in the northern region until 1930 would be mostly migrant population and therefore would show the nine-year cycle only. Later some would become adapted to these regions and stay there and breed. This indigenous population would be under new ecological pressures and would show the native four-year cycle.

In the bush country, any white fox taken should be migrants and show the four-year cycle. This is true, none of the white fox returns show a nine-year cycle. As the colored fox begins to breed in the arctic it builds up

population pressures every four years and migrations southward take place. Such southern migrations should show as supplementary peaks as in the case of the Upper James Bay region in 1942.

## The Coat Color Phases

The evidence for migration obtained from the data by smoothing can be supplemented by evidence from another source. The colored fox exists in three distinct color phases (Butler (3) ) silver or black, cross, and red. These phases are governed in their inheritance by two main pairs of alleles. This being so we should expect the Hardy–Weinberg equilibrium to hold for the genes concerned unless there is differential fertility.

Unfortunately no breeding experiments have been conducted with fox from this area so we cannot be sure whether the coat colors in this region are conditioned by one or two pairs of alleles. The early collections of Quebec wild fox for starting fur farms indicated that only one pair of alleles was present but the data are not extensive enough to be conclusive. Haldane (8) working with earlier data from the same region concluded that selective mating or trapping selection was necessary to explain the data. He did not try two factors. Iljina (9) postulated modifying alleles. Warwick and Hanson as quoted by Ashbrook (2) give a scheme for a pair of interacting alleles. The latter scheme is borne out by limited breeding experiments at the Hudson's Bay Company fur farm. The researches of Butler (3, 4) and Calhoun (5) show how these alleles are distributed throughout Canada and how in many cases the proportions of silver, cross, and red change with different stages of the cycle.

The three phenotypes can be brought about by one pair of alleles or by two pair of alleles. In differentiating between the two modes of inheritance it has been customary to use the Chi square test to determine whether the color phase proportions fit the classic Hardy–Weinberg formula of $p^2 : 2pq : q^2$. If the Chi square has a corresponding $P$ value of less than .05 the single locus theory is usually abandoned and the assumption made that both pairs of alleles are present in the area. The frequency of the four genes is then calculated by the formulae given by Calhoun and no tests of significance are applied to these frequencies. In utilizing these tests of gene frequency it is assumed that the population is panmictic and that no phenotype is favored more than the others.

One of the biggest objections to the application of equilibria formulae to the yearly color phase data has been the apparently wide variations in gene frequency from year to year (Calhoun (5, p. 50) ). These variations occur with a much higher frequency than would be expected by chance alone. This statement is based on tests applied only to years which apparently fit the monohybrid segregation. There were 64 pairs of consecutive years from the various regions and these were tested for homogeneity of the color phase proportions. Twenty-one pairs had Chi squares larger than 5.99 thus indicating a significantly greater amount of change than the three significant differences

between years expected by chance alone. Another method utilized was application of the formulae to runs of years which fit monohybrid ratios and calculation of the variance for each year. The change in gene frequency of consecutive years over the standard deviation gave *P* values which were less than .05 in 12 cases out of 52 tested. This again indicates yearly changes of gene frequency of greater magnitude than would be expected by chance. Long-term trends employed in conjunction with dihybrid inheritance give better fits but are not amenable to statistical tests of significance or to a biological interpretation.

One weakness of the past researches is that no tests of panmixia have been undertaken to determine whether this prime assumption is justified.

## Panmixia Versus Isolates

If the colored fox population for the whole of Quebec is a single breeding population with no barriers to separate it into isolates, then migrations or pulsations due to population pressure will ebb and flow continuously. Under these conditions differences between local populations are not likely to occur. That panmixia has not taken place in this population in the past is seen by evidence from three sources. First, fur graders for years have recognized that different types of fur are produced by various regions whilst domestication on fur farms has shown that such differences are largely hereditary. The fur grader will immediately separate the FG (Fort George) and EM (East Main) type of the James Bay region from the Canada type of Southern Quebec. Fur farm breeding records suggest that the main differences between these two types, and between these and the EB (Eskimo Bay) type from the Labrador border are genetic rather than environmental. The very recognition of such hereditary difference bespeaks of some degree of isolation in the past which must have lasted over a period of several generations in order to accumulate different genotypes.

The above does not infer that all foxes taken, for instance, in the vicinity of Fort George will be of the FG type. Graders reports show that the proportion varies widely from year to year. Unfortunately there is no long series of such reports, the existence of which would form a valuable adjunct to the present study, but two generalizations can be drawn. First that the proportion of pelts of the local type varies from 30-90% in the data of different years. Second, that the type pelts of colored fox are not as easily separated as they were in 1800. This second generalization is not true for other species of fur-bearers.

The second line of evidence is that the taxonomist recognizes differences in the red fox of Quebec and gives them subspecific rank (Anderson (1) ). In the Hudson Bay and Strait regions the Labrador fox *Vulpes fulva bangsi* is found while in James Bay and Southern Quebec the native fox is the Northeastern red fox *V. f. rubricosa*. Extending into the southern and western sections of Quebec we find the Eastern red fox *V. f. fulva*. The recognition of these subspecies suggests some geographic isolation has existed in the past.

The taxonomic data should not be taken to mean that each region is populated by a different subspecies. It must be remembered that no extensive *Vulpes* collections have been made from any locality so that we have no data which will tell us that at one geographic point the collection consists of 100 *V. f. rubricosa* and 20 *V. f. bangsi*. The species distinctions are based on a few pelts collected over the past hundred years from various regions of Quebec. No genetic study has been made of the validity of these subspecies or of the presence or absence of intergrades. The distribution data are based on the fact that single or multiple specimens collected in any area are comparable with such and such a type specimen.

Third, the ratio of the three inherited color phases in a panmictic population should be the same within the limits of random sampling throughout the range. If we group the data according to the homogenicities shown by the cyclic analysis we find that the 1916-28 period gives the ratios shown in Table I.

TABLE I

TEST FOR PANMIXIA OF THE COLOR PHASE RATIOS IN THE YEARS 1916-28

| Region | Color phase, % | | | Total pelts | $\chi^2$ |
|---|---|---|---|---|---|
| | Silver | Cross | Red | | |
| Hudson Strait | 6.4 | 24.7 | 68.9 | 3639 | 16.6 |
| Hudson Bay | 7.0 | 25.0 | 68.0 | 3177 | 27.2 |
| Upper James Bay | 5.8 | 27.4 | 66.8 | 4797 | 59.8 |
| Lower James Bay | 5.5 | 20.4 | 74.1 | 5192 | 18.6 |
| Southern Quebec | 2.0 | 19.5 | 78.5 | 3538 | 268.8 |
| St. Lawrence | 5.5 | 24.7 | 69.8 | 4778 | 9.2 |
| Total | 5.4 | 22.8 | 71.8 | 25092 | — |

The Chi² values in the last column are calculated by using expected pelt numbers based on the ratios shown for the total sections. These Chi² values show that all sections have ratios which could be expected to occur only rarely in a panmictic population, the *P* values in all cases being less than .01. The closest fit was in the case of St. Lawrence section, while the Southern Quebec section was farthest from the provincial average. These Chi² values clearly indicate heterogeneity. This is especially true for the cross phase which shows greater variation than the other two phases. A second hypothesis that Southern Quebec does not belong in this population, but the rest of the sections are panmictic was tested and gave Chi²'s of 1.84, 7.72, 24.6, 49.0, and 1.37. The figures indicate that Upper and Lower James Bay probably do not belong in this population series. A test for agreement between the ratios in the Hudson Bay and Strait sections gave Chi² of 0.53 and 0.61 indicating no real differences in color phase proportions. From these tests

we conclude that we are dealing with at least three isolates, existing in the following regions: Hudson Bay and Strait region, James Bay region, and Southern Quebec. The population is not panmictic.

In the 1916-28 period when only the nine-year cycle appeared to be operating there was a clear north–south gradient in the silver phases. The cross phase shows a similar gradient except for the Upper James Bay region. The Hudson Bay and Strait areas are similar in composition for this period and examination of the data show the composition to be relatively constant for the whole period. The Upper James Bay region has a silver ratio similar to that of lower James Bay but its percentage cross is the greatest of any of the regions.

This cline in color phase percentages indicates that we are dealing with isolates and not a panmictic population. Since this is so the question arises: are they complete isolates or just partial ones, and, if the latter, is the degree of isolation the same each year or does it vary with the population pressures of the surrounding areas? To answer these questions let us make a more detailed examination of the color phase ratios.

In a population which shows a partial breakdown of isolate barriers Wahlund (10) has shown that if a character is brought about by one pair of alleles then the two homozygotic classes will increase in frequency while the heterozygotes (cross fox) will decrease in frequency. The increases and decreases are such that silver and red will each increase Z% while cross decreases 2Z%. This makes possible a different interpretation of some of the ratios because proportions which would not fit ordinary monohybrid expectation on the basis of panmixia will fit when isolates are considered. Applying such formulae to the data in Table I we get the results tabulated in Table II.

The usual method of fitting monohybrid ratios by the use of the formula $r$ = silver + $\frac{1}{2}$ cross gives homozygotes in excess and heterozygotes deficient. In the case of Hudson's Strait in the years 1916-28 we have the following data:

| | Silver | Cross | Red |
|---|---|---|---|
| Actual | 6.4% | 24.7% | 68.9% |
| Expected (from S + ½C) | 3.8% | 31.5% | 64.7% |
| Difference | + 2.6% | −6.8% | + 4.2% |

These differences are suggestive of the isolate effect postulated by Wahlund and it only requires a slight adjustment to get the theoretical ratio 3.5 : 30.5 : 66.0 which gives an excess of 2.9% in both homozygotes (silvers and reds), and a deficiency of 5.8% in the heterozygote cross phase. A theoretical ratio with a slightly higher proportion of silver gives a good fit in the case of Hudson's Bay and Upper James Bay sections. In Lower James Bay the

TABLE II

COLOR PHASE PERCENTAGES IN SIX ISOLATES

| | 1916-28 | | | 1929-33 | | | 1934-38 | | | 1939-43 | | | 1944-48 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | C | R | S | C | R | S | C | R | S | C | R | S | C | R |
| Hudson Strait | 3639† | | | 819 | | | 9156 | | | 5927 | | | 10331 | | |
| Actual ratio | 6.4 | 24.7 | 68.9 | 5.1 | 21.5 | 73.4 | 4.3 | 19.4 | 76.3 | 2.4 | 16.0 | 81.6 | 3.0 | 17.6 | 79.4 |
| Equilibrium ratio* | 3.5 | 30.5 | 66.0 | 2.5 | 26.5 | 71.0 | 2.0 | 24.0 | 74.0 | 2.0 | 24.0 | 74.0 | 2.0 | 24.0 | 74.0 |
| Difference | 2.9 | −5.8 | 2.9 | 2.6 | − 5.0 | 2.4 | 2.3 | −4.6 | 2.3 | 0.4 | −8.0 | 7.6 | 1.0 | −6.4 | 5.4 |
| Hudson Bay | 3177 | | | 439 | | | 3414 | | | 2485 | | | 6407 | | |
| Actual ratio | 7.0 | 25.0 | 68.0 | 5.2 | 28.4 | 66.4 | 3.4 | 20.5 | 76.1 | 3.2 | 20.8 | 76.0 | 3.6 | 19.6 | 76.8 |
| Equilibrium ratio | 3.7 | 31.5 | 65.0 | 3.7 | 31.5 | 65.0 | 2.0 | 24.0 | 74.0 | 2.0 | 24.0 | 74.0 | 2.0 | 24.0 | 74.0 |
| Difference | 3.3 | −6.3 | 3.0 | 1.5 | −2.9 | 1.4 | 1.4 | −3.5 | 2.1 | 1.2 | −3.2 | 2.0 | 1.6 | −4.4 | 2.8 |
| Upper James Bay | 4797 | | | 221 | | | 2234 | | | 1229 | | | 3482 | | |
| Actual ratio | 5.8 | 27.4 | 66.8 | 3.2 | 23.5 | 73.3 | 3.9 | 22.8 | 73.3 | 2.0 | 24.7 | 73.3 | 3.2 | 21.3 | 75.5 |
| Equilibrium ratio | 3.7 | 31.3 | 65.0 | 2.5 | 26.5 | 71.0 | 2.5 | 26.5 | 71.0 | 2.0 | 24.0 | 74.0 | 2.0 | 24.0 | 74.0 |
| Difference | 2.1 | −3.9 | 1.8 | 0.7 | −3.0 | 2.3 | 1.4 | −3.7 | 2.3 | 0.0 | 0.7 | −0.7 | 1.2 | −2.7 | 1.5 |
| Lower James Bay | 5192 | | | 497 | | | 3634 | | | 1568 | | | 8610 | | |
| Actual ratio | 5.5 | 20.4 | 74.1 | 6.4 | 14.0 | 79.6 | 4.0 | 21.2 | 74.8 | 2.9 | 20.5 | 76.6 | 3.7 | 26.1 | 70.2 |
| Equilibrium ratio | 2.5 | 26.5 | 71.0 | 2.0 | 24.0 | 74.0 | 2.0 | 24.0 | 74.0 | 2.0 | 24.0 | 74.0 | 3.0 | 28.6 | 68.4 |
| Difference | 3.0 | −6.1 | 3.1 | 4.4 | −10.0 | 5.6 | 2.0 | −2.8 | 0.8 | 0.9 | −3.5 | 2.6 | 0.7 | −2.5 | 1.8 |
| Southern Quebec | 3538 | | | 605 | | | 5564 | | | 1941 | | | 9646 | | |
| Actual ratio | 2.0 | 19.5 | 78.5 | 2.4 | 9.9 | 87.7 | 1.1 | 11.6 | 87.3 | 1.3 | 12.0 | 86.7 | 2.3 | 16.3 | 81.4 |
| Equilibrium ratio | 2.0 | 24.0 | 74.0 | 2.0 | 24.0 | 74.0 | 1.0 | 18.0 | 81.0 | 1.0 | 18.0 | 81.0 | 1.0 | 18.0 | 81.0 |
| Difference | 0.0 | −4.5 | 4.5 | 0.4 | −14.1 | 13.7 | 0.1 | −6.4 | 6.3 | 0.3 | −6.0 | 5.7 | 1.3 | −1.7 | 0.4 |
| St. Lawrence | 4778 | | | 1278 | | | 3939 | | | 1828 | | | 3406 | | |
| Actual ratio | 5.5 | 24.7 | 69.8 | 5.2 | 21.8 | 73.0 | 6.4 | 21.3 | 72.3 | 3.2 | 19.4 | 77.4 | 3.5 | 18.0 | 78.5 |
| Equilibrium ratio | 3.0 | 28.6 | 68.4 | 3.0 | 28.6 | 68.4 | 3.0 | 28.6 | 68.4 | 2.0 | 24.0 | 74.0 | 2.0 | 24.0 | 74.0 |
| Difference | 2.5 | −3.9 | 1.4 | 2.2 | −6.8 | 4.6 | 3.4 | −7.3 | 3.9 | 1.2 | −4.6 | 3.4 | 1.5 | −6.0 | 4.5 |

** Based on partial breakdown of isolate barriers—see text.* *† Total number of pelts.*

use of the theoretical ratio 2.5 : 26.5 : 71.0 brings this into line with differences of + 3.0% S : − 6.1%C : 3.1%R. The St. Lawrence section is also amenable to such reasoning and the only one which will not fit is Southern Quebec. In this section we find that all theoretical ratios based on Hardy–Weinberg equilibrium for monohybrid inheritance give a deficiency of both silvers and cross and an excess of reds.

An interpretation of the cycle and genetic data for this period would be that in all regions except Southern Quebec there existed fox populations with a sufficient degree of isolation to develop different proportions of the coat color phases. With the rhythmic population increases isolate boundaries were broken and the resulting populations showed the excesses of homozygotes and a deficiency of heterozygotes expected under such conditions. This appears to be a much better explanation than the differential selection postulated by both Haldane and Calhoun, and is an extension of the migration theory put forward by Butler. In the case of both Calhoun and Butler they were seeking to explain why in many cases the proportion of one color phase fluctuated with the population size. If we accept the isolate theory then the isolate-breaking effects of migration will explain satisfactorily the sudden shifts in gene frequency. The long term trend can also be explained by this mixing of isolates. The largest expansion of population has come in the south where the silver frequency is low and the red frequency high. The two or three northward expansions have reduced the northern silvers from 7.0 to 2.5% and at the same time have increased the percentage reds from 68.0 to 76.0%. In Southern Quebec there has also been an increase of from 6-8% in reds and possibly a slight decrease in the silvers. The change in silvers may not have been a permanent one.

In the case of the breaking down of isolate barriers and the mixing of two or more isolates with different gene frequencies we would expect the homozygotes red and silver to increase or decrease in proportion to the mixture. In the north for instance we would expect an admixture with a southern isolate to increase the percentage of reds and decrease the silvers while in the south the reverse should be true.

This is shown in Fig. 6 where the population data and percentage reds is plotted for the combined Hudson's Bay and Strait sections. From 1916 to 1928 there is an almost perfect correlation between population changes and fluctuations in the percentage of the red phase. From 1929 – 33 there is some correlation shown except in the year 1933. In this year there is an increase in the percentage of red and a decrease in the population. The actual data for this year are based on 141 pelts and has an anomalous cross percentage as can be seen by comparing it with the two previous and two succeeding years, the percentage cross phase in these five years being 26.6, 24.5, *17.0*, 23.9, and 21.5. The 5% or more which is added to the red phase by this decrease in the cross phase is enough to cause lack of correlation in this particular year. From 1934 – 42 there is a positive correlation but it is not as marked because the previous influx of southern foxes has reduced the

differences between the isolates. From 1943 to 1948 there is no correlation. The increase in population has been brought about either by the reproduction of the local population or else by the influx of foxes with a similar percentage of the red phase. It should be noted that the increase trend in population is closely paralleled by the increase trend in the red phase.

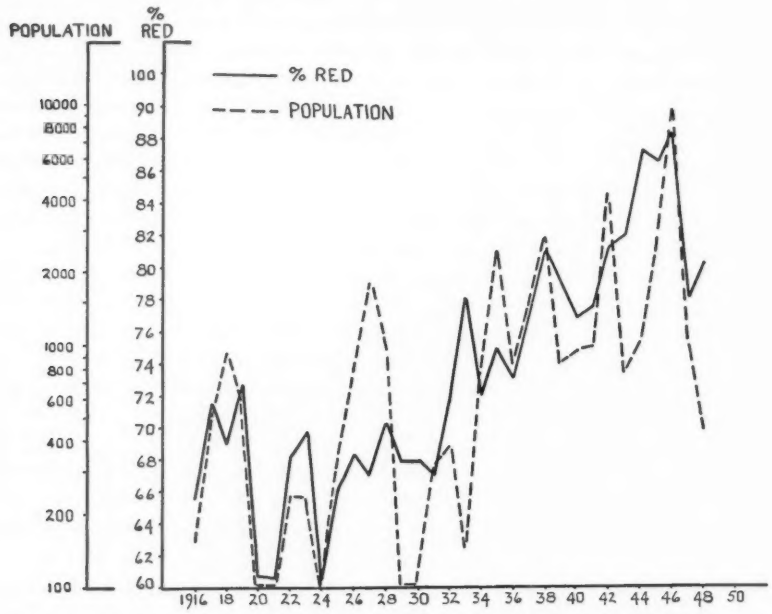


FIG. 6. Yearly pelt collections and percentage reds in Hudson Bay and Strait region.

Wahlund gives a formula for calculating the respective amounts of each isolate in the breeding population. This formula is based on the standard deviation but in this case we are dealing with so few isolates that no accurate estimate could be made.

The difference in interpretation possible by accepting partial isolation instead of panmixia can be seen from Table III. In this table the $\chi^2$'s are calculated on the assumption of panmixia and monohybrid equilibrium, and it will be seen that the smallest $\chi^2$ is 78.4 indicating that this hypothesis certainly does not fit; $P_{.01}$ being 9.1. If on the other hand we accept partial isolation and calculate ratios based on equilibrium formula modified by equal increases in the reds and silvers balanced by a decrease in the cross phase we get the $\chi^2$'s given in the last column. In this case another degree of freedom has been lost by equalizing the silver and red excesses but except in the case of Southern Quebec we do not have to resort to the theory that both sets of alleles are present.

TABLE III

TEST FOR MONOHYBRID INHERITANCE OF COLOR PHASES

(DATA FROM TABLE I AND FIRST COLUMN TABLE II)

| Region | $\chi^2$ equilibrium | $\chi^2$ isolates |
|---|---|---|
| Hudson Strait | 131.2 | 0.04 |
| Hudson Bay | 167.6 | 0.84 |
| Upper James Bay | 78.4 | 0.85 |
| Lower James Bay | 265.1 | 0.14 |
| Southern Quebec | 106.4 | 351.6 |
| St. Lawrence | 124.3 | 2.30 |

In Table II we have not tried to get the ratio which gives the best fit but instead we have put down generalized ratios which call for only minor changes in the gene frequency from one period to the next. These changes are in the direction which would be expected with the breaking of the isolate barriers and northern migration. Since it appears that the Alaskan gene is also present in the Southern Quebec region it is to be expected that it would spread northward through the other regions and modify these ratios. As far as we are concerned in the present paper it does not matter whether the changes in ratios are brought about by the mixing of one or two sets of alleles since the overall effect we wish to emphasize is that we are dealing with isolates and that migration does take place between these isolates.

## Discussion

The data presented show that there is a nine-year periodicity in the southern regions and a four-year cycle in the northern regions. The boundary between the nine- and four-year cycles is not clear-cut. There is definite evidence of a four-year cycle peak in 1942 in the Upper James Bay region which normally shows only nine-year periodicity. Furthermore in the area north of the timberline which has a four-year cycle in white fox and lemming the colored fox at first shows a nine-year cycle and then changes over to a four-year cycle. Close inspection, however, shows that the four-year cycle which develops has the former nine-year cycle concealed in it. The coexistence of both cycles in this one region raises a point of further interest.

In the theory of population cycles there have been two theories as to the cause of peak populations. One has held that breeding conditions and chances of survival in that particular region have been particularly good so that the *local* population has increased to peak numbers. While the other theory has held that animals have moved in from the surrounding regions creating a peak population. The second theory has been largely abandoned because Elton showed that the peak populations occur at approximately the same time all across the country. This being so, there would be no place from which the animals could come in order to give simultaneous country-wide

immigration. There is some evidence that the peak years are not identical all across Canada but are such that they could be brought about by waves of migration spreading across the country from several foci of increase. With these points in mind let us examine the Quebec red fox data.

The cycle and color phase analyses for the Hudson Strait and Bay regions show that there is a nine-year cycle in the data, and that most of these foxes are immigrants from the south. Later, more foxes bred in this region and the local ecological conditions affected these so that they showed the four-year cycle. We have a measure of the local food conditions in the state of the lemming population as given by Chitty (6). Comparing the nine-year peaks in 1927, 1937, and 1946 with the lemming data we find that each peak occurred at a different stage in the lemming cycle. The first one came at the peak of the lemming population, and the rise and fall of both populations is parallel. The 1937 peak came with the first year of increase in the lemming cycle and the 1946 peak corresponds to the first year of decrease in lemmings. In both 1927 and 1946 the fox moved into the region when there was plenty of food present. In 1937 while the food supply was increasing there was an abundance of local foxes present from the preceding four-year peak so that the situation was not attractive to migrants. In spite of this they moved in. Thus it appears that the migrations we have demonstrated are the result of population pressure in the home area becoming so great that many are forced out. These take the path of least resistance which is generally northward. This would explain the simultaneous increase in both the red fox population and the northern range.

Generalizing from the foregoing we would say that the secondary cause of a cycle can be classified in three categories:

1. An expansion of the local population brought about by either an increased birth rate or increased survival. Most evidence points to the latter factor. Under such conditions the population becomes so dense that competition for food and home ranges often forces part of the population to migrate.

2. A certain species is scarce or absent in the locality and individuals fleeing from population pressure elsewhere move in. Under these conditions the population cycle is liable to show little correlation with local conditions.

3. A combination of 1 and 2. There is a local population which is fluctuating but is still sparse enough to offer less resistance to immigrants than other regions adjacent to the area with overpopulation.

In the latter case if the population in both areas is showing the same type of periodicity and in the same phase then there is usually no way of telling what proportion of the amplitude is due to the local population. This can be done in the case of the colored fox by the relative changes in the coat color phases. When the cycle is not of the same length in the two regions then the area may either show a cycle length not typical for that region or else a mixture of the two cycle lengths.

If we accept these three secondary causes of population cycles we come to the conclusion that the primary causes are located within the specific regions. The conditions south of the timberline are such that many of the furbearers show a nine-year periodicity, while north of the timberline they show a four-year periodicity. The primary causes are not within the animal itself but rather within the environment in which it breeds and raises its young. Thus a fox breeding south of the timberline will tend to conform to the nine-year cycle but the same fox breeding north of the timberline will tend to conform to the four-year cycle. Migrations will correspond in cycle with that in the area from which they emigrated.

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# THE EFFECT OF INSECTICIDES ON THE RATE OF OXYGEN CONSUMPTION IN *BLATTELLA*[1]

By G. T. Harvey[2] and A. W. A. Brown[3]

## Abstract

The effect of 26 insecticidal compounds upon the rate of oxygen consumption of the German cockroach was ascertained by their injection into adult male insects at the approximate $LD_{50}$ level. The chlorinated hydrocarbons, dinitro compounds, pyrethrins, nicotine, azobenzene, and the organic phosphates had a pronounced stimulatory effect upon this rate. With the chlordane compounds, also aldrin and dieldrin, this effect was preceded by a long latent period. Ryania and rotenone were depressant after a slight initial raising of the rate of respiration, while sabadilla gave a slight rise but was not depressant. Phenothiazine had little or no effect; and the organic thiocyanates were entirely depressant. Similar results were obtained by spray application of these insecticides.

## Introduction

The present study is designed to shed some light upon the manner in which the great variety of insecticides now available exert their lethal effects. Past research in this field, as shown by a recent survey (11), has revealed more about the mode of entry of poisons into the insect than their toxic action on the tissues and organs. In the experiments reported below, the rate of oxygen uptake of the intact insect was measured as a quantitative expression of the total physiological effect of a series of important organic insecticides. Following initial experiments with contact sprays, the insecticides were injected directly into the body cavity in order to avoid factors involved in the mode of entry.

## Material

Adult males of the German cockroach (*Blattella germanica* L.) were used as the test insects, being taken between one week and two months after the final molt.

Their body weight averaged 54.9 mgm., with a standard deviation of 6.9 mgm. Full particulars of the 26 insecticides employed are set out in Table I. They were dissolved in corn oil (Mazola, Canada Starch Co.) before injection. The dosages employed are shown in the last column of the table; a dose of 10 $\mu$gm. corresponds to 1 cu. mm. of a 1% solution. Dosages of commercial preparations are based on the pure compound present, and those of ryania and sabadilla on the amount of the ether extract. Water was used as the solvent for nicotine alkaloid.

[1] *Manuscript received in original form May 22, 1950, and as revised, August 30, 1950.*
*Contribution from the Department of Zoology, University of Western Ontario, London, Ont. This investigation was financially assisted by a grant-in-aid from the National Research Council of Canada.*

[2] *National Research Council Studentship holder.*
[3] *Professor of Zoology, University of Western Ontario.*

TABLE I

PURITY, SOURCE, AND FULL DESIGNATION OF INSECTICIDAL MATERIALS INJECTED INTO *Blattella*

| Insecticide | Fuller designation | Purity | Source | Dosage, μgm./roach |
|---|---|---|---|---|
| DDT | *p,p'*-Dichlorodiphenyltrichloroethane | Purified | Experimental Stn. Suffield* | 100 |
| Methoxychlor | *p,p'*-Dimethoxydiphenyltrichloroethane | Technical | DuPont Experimental Station | 100 |
| Lindane | gamma-Hexachlorocyclohexane | Purified | Experimental Stn. Suffield* | 1 |
| Toxaphene | Chlorinated Camphene | Technical | Hercules Powder Co. | 100 |
| Chlordane | The mixture 'Technical Chlordane' | Refined | Julius Hyman & Co. | 10 |
| α-Chlordane | *cis*-Octachlorohexahydromethanoindene | Purified | Julius Hyman & Co. | 10 |
| β-Chlordane | *trans*-Octachlorohexahydromethanoindene | Purified | Julius Hyman & Co. | 10 |
| Heptachlor | Heptachlorotetrahydromethanoindene | Purified | Julius Hyman & Co. | 10 |
| Aldrin | Hexachlorodiendomethanohexahydronaphthalene | Purified | Julius Hyman & Co. | 10 |
| Dieldrin | Epoxy derivative of Aldrin | Purified | Julius Hyman & Co. | 10 |
| DNOC | 3,5-Dinitro-ortho-cresol | Technical | Standard Agric. Chemicals | 10 |
| DNCHP | 2,4-Dinitro-6-cyclohexylphenol | Purified | University of Toronto | 10 |
| DN-289 | 4,6-Dinitro-2-sec-butylphenol** | Technical | Dow Chemical Co. | 100 |
| Nicotine | White Label | Purified | Eastman Kodak Co. | 100 |
| TEPP | Tetraethyl pyrophosphate | 40% in phosphates*** | Monsanto Chemical Co. | 1 |
| Parathion | Diethyl-*p*-nitrophenyl thiophosphate | Technical† | American Cyanamid Co. | 1 |
| PDB | *p*-Dichlorobenzene | Purified | Eastman Kodak Co. | 100 |
| Dichloroethyl | (β,β'-Dichlorodiethyl) ether | Purified | Dow Chemical Co. | 100 |
| Pyrethrins | Pyrefume Super 100 | 12% in kerosene | S. B. Penick & Co. | 100 |
| Rotenone | From ground *Derris* root | Purified | University of Toronto* | 100 |
| Ryania | From *Ryania speciosa* plants | Ether extract | Merck & Co. | 100†† |
| Sabadilla | From 50% *Sabadilla* seed | Ether extract | McConnon & Co. | 100†† |
| Phenothiazine | Thiodiphenylamine | Technical | Dow Chemical Co. | 100 |
| Azobenzene | White Label | Purified | Eastman Kodak Co. | 200 |
| Lethane 60 | Thiocyanoethyl laurate | 50% in kerosene | Rohm & Haas Co. | 100 |
| Lethane 384 | β-Butoxy-β'-thiocyanodiethyl ether | 50% in kerosene | Rohm & Haas Co. | 100 |

* *Purified by Dr. D. B. W. Robinson, Mr. W. W. Hopewell and Prof. G. F Wright respectively.* ** *Triethanolamine salt.* *** *Nifos-T.*
† *Thiophos 3422.* †† *100 μgm. of the ether extract.*

## Methods

Oxygen consumption was measured by a Warburg–Barcroft respirometer, using constant volume manometers and 15 ml. sidearm flasks each containing one insect. Carbon dioxide was removed as soon as it was produced, by placing 0.4 ml. of 20% potassium hydroxide solution and a few strips of filter paper in the center well of the flask. The roach was confined to the moat surrounding the well, by a doughnut-shaped disc of plastic screening. Experiments were performed in triplicate, at a temperature of 27° C., and three control insects were simultaneously injected with corn oil alone; a seventh manometer served as a thermobarometric control (14).

The insects were injected through the intersegmental membrane behind the fifth abdominal ventrum, using a standard No. 27 hypodermic needle and a 0.25 ml. syringe mounted in line with a micrometer (Starrett No. 263). They had been immobilized with carbon dioxide anesthesia and kept at 32° C. until injected; this treatment had no permanent effect on the subsequent respiration. The corn oil solvent had no appreciable effect on the respiratory rate beyond a slight initial depression (Fig. 1).

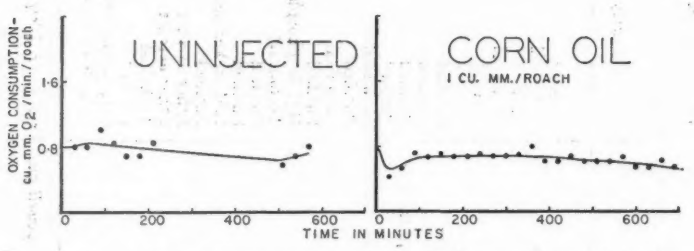


FIG. 1. Rate of oxygen consumption in the German cockroach, both in normal untreated condition and after injection with 1 cu. mm. of corn oil.

The flask and its contents were quickly equilibrated for temperature and measurements were commenced within 15 min. of injection. Manometer readings were taken every 10 min. thereafter until the insects had become completely motionless, the experimental period being in most cases 700 min. The dosages employed were selected on the basis of the relative contact toxicity of the insecticides as already determined for *Blattella* (1), and were at one of three levels, either 1, or 10, or 100 $\mu$gm. per individual roach. Where contact toxicity data were not available, the correct injection dosage was established by direct test to find that which gave a suitable period of time to death.

## Results

The effects of the various insecticides upon the respiratory intake are presented in graphical form in Figs. 2 to 8.

Each graph represents the oxygen consumption of a single typical insect. This was selected from three, six, or nine individuals whose respiration was

measured for each compound tested. The values of the abscissae denote the elapsed time after injection; groups of three 10 min. increment readings have been averaged together to give the average rate of oxygen consumption over a 30 min. period. The initial value for oxygen consumption was taken from the mean level shown by the three control insects injected with corn oil alone. The average rate of oxygen consumption for all the controls studied was 0.69 cu. mm. per min. per individual, corresponding to 12.6 cu. mm. per min. per gram wet weight, or 44.4 cu. mm. per min. per gram dry weight of tissue.

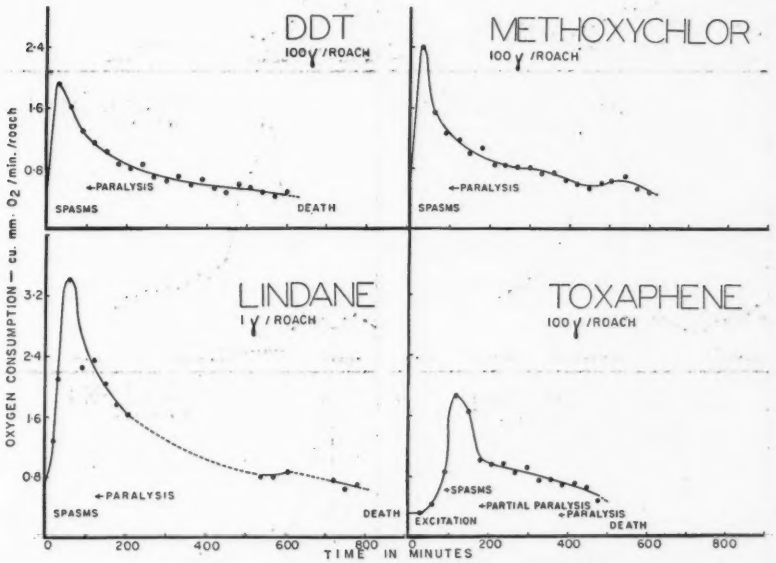


FIG. 2. Rate of oxygen consumption of the cockroach injected with certain chlorinated hydrocarbon insecticides at the dosages cited.

The results obtained with the chlorinated hydrocarbon insecticides are characterized by a rapid rise in oxygen consumption (Figs. 2 and 3). In the case of DDT and methoxychlor (Fig. 2), the increase occurs immediately after injection, and reaches a peak within 30 min. With lindane the respiratory rate continues to rise for a longer period, to reach a peak in 60 min. when it is five times the normal. All three compounds caused paralysis to appear about 60 min. after the peak of respiration, halfway on its return to the normal level. With toxaphene the threefold increase is preceded by a latent period of 60 min. duration, and the paralysis commencing one hour after the peak of respiration is incomplete. Insects under the influence of

these insecticides consumed no more than 15% of the oxygen in the air contained in the flask; therefore oxygen lack does not become a limiting factor as it did in the experiments of Buck and Keister (2) with DDT on *Phormia*.

The "chlordane compounds" (Fig. 3) are characterized by a long latent period followed by an abrupt rise in respiration. With heptachlor the latent period averages 150 min., and the peak of respiration is about five times the normal level. The latent period is very greatly prolonged in the case of

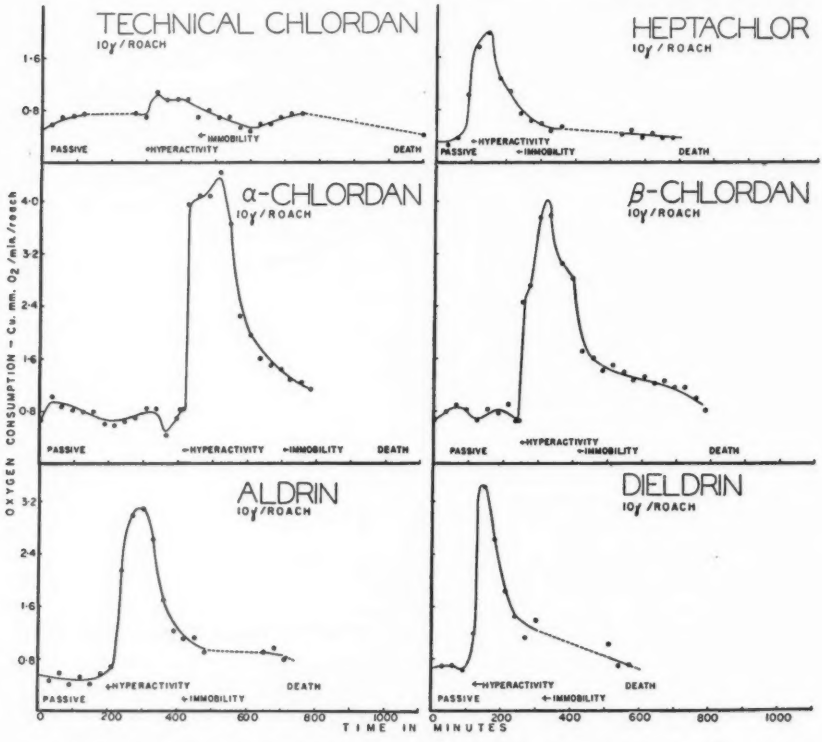


FIG. 3. Rate of oxygen consumption of the cockroach injected with certain of the "chlordane compounds" and with aldrin and dieldrin.

α-chlordane and β-chlordane, which cause a sixfold increase after latent periods of at least 400 min. and 250 min. respectively. When technical chlordane is employed, the increase is much reduced, the peak being no more than twice that of normal, after a 300-min. latent period. Both aldrin and dieldrin cause a fivefold increase in respiration; the latent period was found to be 200-300 min. for aldrin and 100 min. for dieldrin.

The chlorinated insecticides PDB (*p*-dichlorobenzene) and dichloroethyl ether show curves similar to those of DDT, causing a three- to four-fold

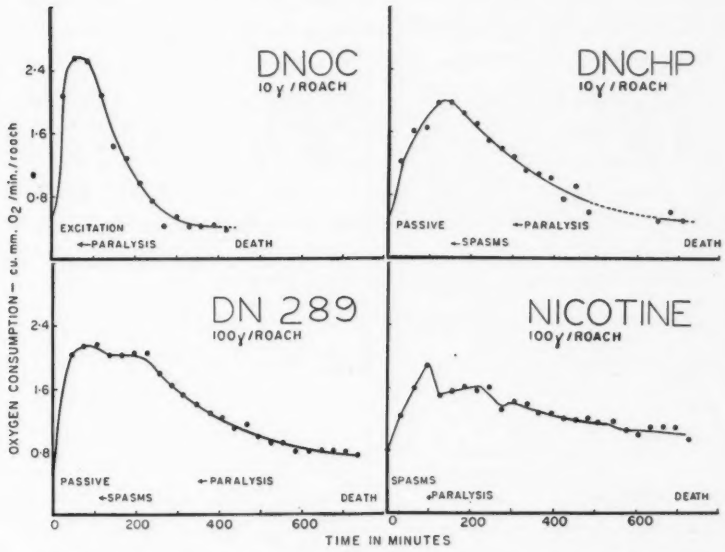


FIG. 4. Rate of oxygen consumption of the cockroach injected with certain dinitrocompounds and with nicotine.

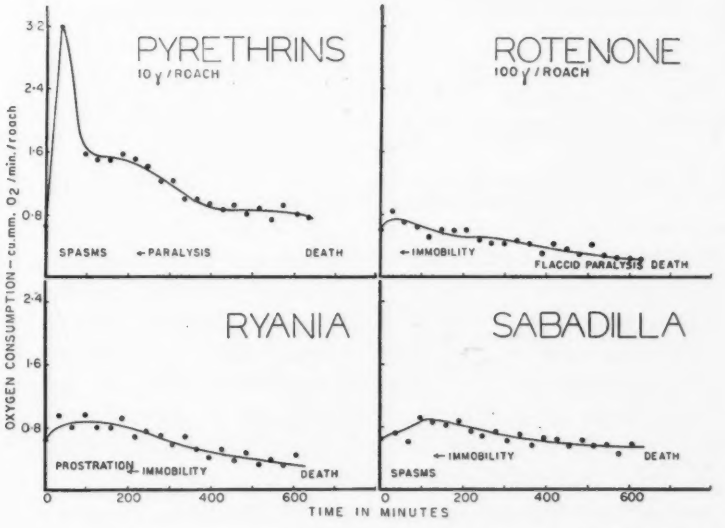


FIG. 5. Rate of oxygen consumption of the cockroach injected with certain insecticides derived from plants.

increase without a latent period. The irregular decrease of oxygen consumption shown for PDB is characteristic, and is associated with irregular clonic spasms (Fig. 6).

The dinitro compounds also cause a marked initial increase in respiration, being most pronounced with DNOC and least so with DNCHP (Fig. 4). The organic phosphates also induce a respiratory stimulation of about three times the normal (Fig. 6). There was a latent period of 60 min. before parathion took effect; with TEPP the stimulation appeared immediately.

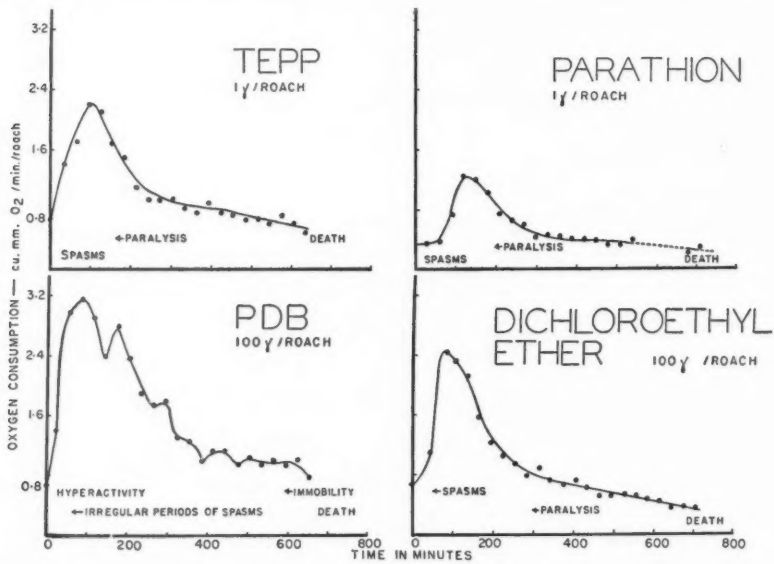


FIG. 6. Rate of oxygen consumption of the cockroach injected with certain organic phosphates, PDB, and dichloroethyl ether.

The pyrethrin preparation employed caused an immediate and pronounced increase of respiration which was as transient as it was rapid; paralysis set in three hours later (Fig. 5). With nicotine, there was a more gradual rise to a peak about one-half as pronounced as that of the pyrethrins; the subsequent fall was irregular, since the paralysis was broken periodically by slight movements (Fig. 4). The last compound to fall into the class of respiratory stimulants was azobenzene, which caused a gradual rise until the oxygen consumption was three times the normal rate (Fig. 7).

The botanical extracts of ryania and sabadilla constitute a separate class in that they cause only a slight increase in respiration during the initial period of intoxication (Fig. 5). The maximum increase was 40% that of normal

in either case, and the level returned to normal within 300 min., thereafter to decrease steadily and gradually. These materials caused immediate prostration, which might be accompanied by spasms which were more frequent in the case of sabadilla poisoning. Rotenone also induced only a slight increase in respiration, which is so transient that respiration has returned to normal in 100 min. and thereafter it steadily decreases, the insect becoming motionless within the first hour.

A third class of compounds is exemplified by the Lethane insecticides (organic thiocyanates), which cause an immediate and continuous depression of the rate of oxygen consumption (Fig. 7). The general depression is stronger

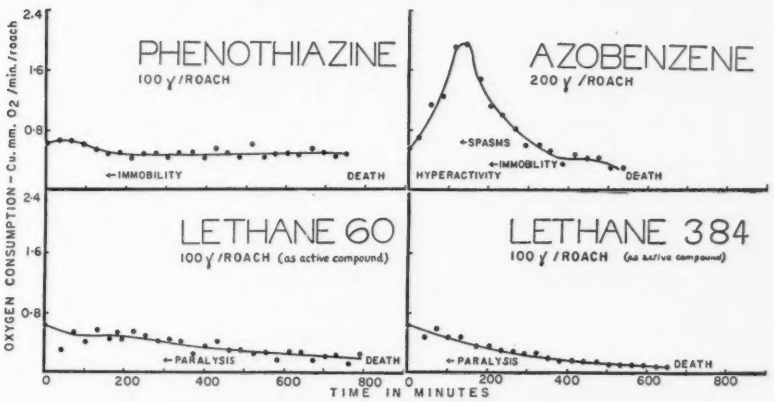


FIG. 7. Rate of oxygen consumption of the cockroach injected with certain lethane thiocyanates, phenothiazine, and azobenzene.

and paralysis sets in earlier in the case of Lethane 384 ($\beta$-butoxy-$\beta'$-thiocyanoethyl ether) than with Lethane 60 (thiocyanoethyl laurate). Phenothiazine is remarkable in having no initial effect whatsoever on respiration (Fig. 7). The insect becomes motionless and the respiratory rate continues steadily at a level slightly below the normal. During this flaccid paralysis the body tissues became noticeably darker.

In general, similar results were given by spray application of the insecticides as by injection (Fig. 8). DDT, lindane, DNOC, and pyrethrins proved highly stimulatory, while Lethane 60 was depressant and rotenone caused a slight transient rise in respiratory rate. As might be expected, the initial increase in oxygen consumption on spray application of DNOC and pyrethrins was more gradual than when they were injected. In the case of DDT, there was a definite latent period of 60 min.; this period was lacking with lindane (a fumigant of low vapor pressure), which caused the peak to be reached 30 min. after spraying.

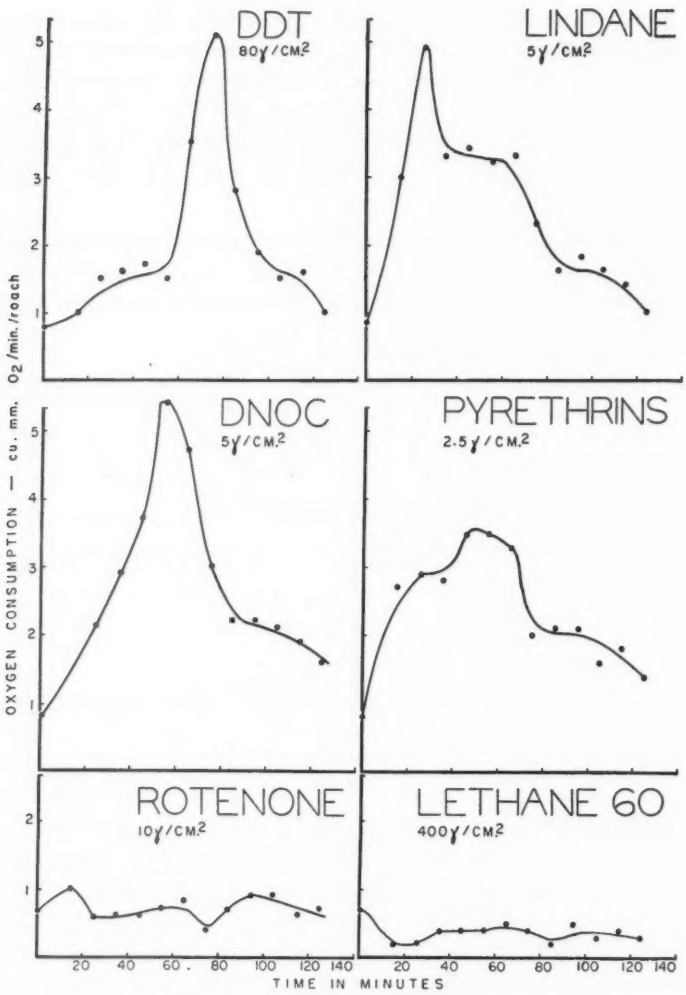


FIG. 8. Rate of oxygen consumption of the cockroach sprayed with certain insecticides at the Area Deposits cited.

## Discussion

These results are substantially in agreement with data reported by other workers. The stimulating effect of DDT in increasing oxygen uptake has been reported by Lord for adults of *Oryzaephilus* (8) and *Tribolium* (9), by Buck and Keister (2) for *Phormia* adults, and by Ludwig (10) for *Popillia* larvae. Methoxychlor has also been found to stimulate the respiration of *Oryzaephilus* (8), and lindane has been found to cause a rise in respiratory

rate of both *Oryzaephilus* (8) and *Tribolium* (9). The latent period, observed in these experiments before any rise in respiration occurred under the influence of chlordane or toxaphene, is also recorded in their effect on *Tribolium castaneum* (9).

The strong acceleration of respiration found in *Blattella* treated with dinitro compounds confirms the results of Goble and Patton (5), who found sublethal doses of these insecticides to increase the respiration of *Apis* adults; and of Lord (8, 9), who found that DNOC caused a rapid initial rise in the oxygen consumption of *Tribolium* adults. The initial stimulatory effect of pyrethrins, well marked in the case of *Blattella*, had also been noted for *Oryzaephilus* (8) and *Tribolium* (9).

The experiments with *Blattella* have shown that the organic phosphates stimulate respiration, the effect being immediate with TEPP but delayed in the case of parathion. Lord (9) has shown that this latent period occurs in the effect of parathion (but not of HETP) on *Tribolium*, and that the length of the period is inversely proportional to the concentration applied.

The predominantly depressant action of rotenone has also been reported for the respiration of *Oryzaephilus* (8). It has been observed by Tischler (13) that the rotenone-bearing derris dusts halted the movements of the tracheal opercula in *Bombyx* and *Heliothis* larvae. The slight increase in respiration with sabadilla has also been noted by Cole and co-workers* for *Oncopeltus* adults treated with contact dusts. The depressant action of thiocyanates has been reported by Lord (8) in the effect of Lethane B-71 on *Oryzaephilus* (8), and of lauryl thiocyanate on *Tribolium* (9).

The results obtained with ryania extract on *Blattella*, namely a slight respiratory increase not exceeding 40% of normal, is not quite in agreement with those of Edwards *et al.* (4), who found that sublethal doses of ryanodine, the active principal of ryania, doubled the rate of oxygen consumption by *Periplaneta* and *Bombyx*.

It is noteworthy that all the insecticides which stimulated the respiratory rate of the unconfined insect, such as the chlorinated hydrocarbons, the dinitro compounds, nicotine, pyrethrins, and the organic phosphates, are known to be nerve poisons of some kind or other. It is probable that the increase of respiration to a peak is an expression of the initial excitatory phase, which in the case of pyrethrins has been attributed to the stimulation of the peripheral sensory nerves (7). The chlordane compounds, aldrin and dieldrin, are aberrant in involving a long latent period, and, in the case of technical chlordane, the stimulation is very weak when it finally occurs. This may be related to the finding of Roeder (12) that technical chlordane has no effect on synaptic transmission of impulses in the nerve preparation of *Periplaneta*, and to the experience in mammalian toxicology that chlordane caused no neurologic disturbances, but only systemic and liver poisoning.

* *Material not yet published, by E. L. Cole, W. H. McShan, and J. H. Lilly.*

These experiments show that ryania resembles rotenone in depressing the respiratory rate after a slight initial increase. It has been found by Edwards *et al.* (4) that ryanodine inhibits the contraction of insect muscle; it thus resembles rotenone in causing a flaccid paralysis. The immobilization of the insect by phenothiazine, observed in these experiments, was originally noted in *Chaoborus* larvae (3), which could remain immobile for three weeks with the heart still beating. The depressant effect of the thiocyanates on respiration is consistent with the observation of Hutzel (6) that Lethane 384 rapidly paralyses *Blatta* without any initial activation.

## Conclusions

The following insecticides have a marked initial stimulatory effect on the rate of oxygen consumption of the German cockroach when injected into the unconfined insect: the chlorinated hydrocarbons, the dinitro compounds, pyrethrins, nicotine, azobenzene, and the organic phosphates. A prolonged latent period precedes the sudden increase in respiration caused by the chlordane compounds, aldrin and dieldrin. Ryania and rotenone have a depressant effect on respiration after an initial slight increase, while sabadilla causes a slight increase without the depressant effect. Phenothiazine has virtually no effect on the respiratory rate. The organic thiocyanates have an immediate and continuously depressant effect on the rate of oxygen consumption. Similar results are obtained when the insecticides are applied by spraying as by injection.

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# THE EFFECT OF INSECTICIDES ON THE HEARTBEAT OF *PERIPLANETA*[1]

By W. B. Orser and A. W. A. Brown


## Abstract

The effect of nine insecticidal compounds upon the rate of heartbeat of the American cockroach was ascertained by their injection into adult male insects at a dosage of 80 μgm. per individual, corresponding to 100 mgm. per kgm. body weight. The chlorinated hydrocarbons DDT, lindane, chlordane, and toxaphene exerted little effect, the maximum response being a slight acceleration. The dinitro compounds DNOC and DNCHP caused an increase in rate, followed by total failure of the heart. Parathion and thiocyanoethyl laurate proved to be slightly stimulatory in the concentrations employed. Rotenone steadily depressed the rate of heartbeat until it stopped. Similar effects were produced by applying the insecticide in a contact spray in benzene–kerosene mixture, although the solvent caused an initial drop in heart rate that was probably associated with temporary narcosis. The heart of decapitated insects showed a gradual decrease in pulsation rate and was less sensitive to the effects of insecticides.


## Introduction

In this investigation the rate of pulsation of the dorsal vessel or "heart" of the insect was taken as a convenient quantitative criterion of the physiological effect of certain organic insecticides. The most recent view of the heart mechanism of arthropods is that it is neurogenic and controlled by a cholinergic accelerator and an adrenergic pacemaker, the latter differing from that in the vertebrate heart (6). Since in insects the blood circulation is not necessary for respiratory exchange, heart failure is not as crucial as it is in vertebrates.

In the American cockroach *Periplaneta americana* (L.), the heart extends from its blind end in the eighth abdominal segment forward to the first thoracic segment, with more or less distinct swellings and a pair of vertical ostia in each segment (2). It is dilated in diastole by the contraction of the alary muscles by which it is supported from the dorsum and is pulsed into systole by the contraction of circular striated muscle fibers embedded in its walls. The heart and its alary muscles are innervated from two sources: (i) The paired segmental cardiac nerves which ascend dorsally from the ganglia of the ventral nerve cord, and (ii) a pair of lateral nerves that run the full length of the heart and arise from the stomatogastric ganglion (1).

The following studies were made by applying moderate lethal doses of insecticides to the intact insect either by injection or as a contact spray. The change in rate was observed on the resting insect, and compared with that of

[1] *Manuscript received August 31, 1950.*
*Contribution from the Department of Zoology, University of Western Ontario, London, Ont.*
*This investigation was financially assisted by a grant-in-aid from Science Service, Department of Agriculture, Ottawa.*

the same specimen, at rest before the treatment. A parallel series of treatments was made on insects which had been decapitated, in order to minimize the effect of the central nervous system, and the general tonus of the body, on the heart.

## Material

The biological material consisted of adult males of the American cockroach, *Periplaneta americana* (L.),* reared in the laboratory at 80° F. and 60% R.H. on compressed meat–vegetable pellets with yeast supplement. Their average body weight was 800 mgm. The chemicals employed consisted of nine insecticidal materials: DDT, lindane, DNOC, DNCHP, and rotenone were purified crystalline compounds; toxaphene, chlordane, and parathion were technically pure; and thiocyanoethyl laurate was in a commercial preparation. Details are given in Table I of the preceding paper (5). For injection into the roaches, 0.8% solutions of these materials were made in corn oil (Mazola, Canada Starch Co.). For direct contact spraying of the insects, the insecticides were dissolved in a mixture of one part of benzene (reagent grade) and two parts of odorless kerosene (Shell Oil Co.).

## Methods

Preliminary to injection two cockroaches were each secured to a small square plaque of waxed cardboard by passing two micropins through the sides of the pronotum and one through the venter of the last abdominal segment and their wings were removed with microscissors. One of the pair was decapitated by ligating its neck with a silk thread, severing the head with microscissors, and sealing the stump with molten paraffin (55° C.).

They were placed in a glass tube 7 in. long and 1.5 in. in diameter, which was held in a plastic trough in the stage of a binocular microscope. Air was drawn through this tube at 80° F., and a water-cell was interposed to cool the radiation from the spotlight type of microscope lamp that was used for illumination. After an interval of 15 min. to allow the insects to become quiet, the rate of their heartbeat was measured visually under the microscope with the aid of a stopwatch and a hand tally. A period of one minute was employed for each count, and 10 such counts were made to determine the pretreatment rate of pulsations. The heartbeat of an intact insect was found to continue at a reasonably constant rate provided the temperature did not change and there was no sudden visual or mechanical stimulus. However, there was considerable variation among individuals, some showing a rate of 130 per minute, others 70 per minute, with the majority beating at 90 to 110 per minute when the temperature was 80° F.

Each insect was then injected with 10 cu. mm. of the solution of insecticide in corn oil, by means of the syringe and micrometer already described (5). The dosage was 80 μgm. (gamma) per roach, corresponding to 100 μgm. per gm.

* *Courtesy of Gulf Research and Development Co., Cheswick, Pa., through Dr. Arnold Mallis.*

or 100 mgm. per kgm. body weight. The needle was introduced through the conjunctiva between the seventh and eighth abdominal terga, and close to the lateral border so as to avoid any mechanical effect on the heart. The treatment caused the insect to exhibit a series of writhings and to become hypersensitive to stimuli, but this effect disappeared within one minute. Counts of the heart beat were then made at as close intervals as possible for the first 10 min., and then at gradually lengthening intervals for the next 90 min. The procedure for each insecticide was replicated three times, involving six insects in all.

Attempts were also made to develop an aqueous injection fluid instead of the oil base; all proved unsuccessful. The emulsion of Roeder and Weiant (7), composed of 20% medicinal paraffin emulsified in insect saline with triethanolamine – oleic acid by means of a Waring blender proved to have a radical effect on the heart independent of any dissolved insecticide. When 10 cu. mm. of this emulsion was injected, the heart immediately showed a great decrease in frequency and amplitude of beat. After a transient period of gradual recovery, complete arrest supervened within five minutes, first appearing in individual segments and eventually spreading throughout the entire organ; the globules of oil were found to have accumulated in the lumen of the heart. The use of acetone suspensions, in which the insecticide is dissolved in acetone which is then diluted with saline, was a failure because of the toxicity of the acetone; an injection of 10 cu. mm. of 1% acetone proved to decrease the heart rate by 20%. The substitution of the nontoxic propylene glycol, a 5% solution of which does not affect the heart rate, was unproductive owing to its poor solvent properties for most of the insecticides. Cyclohexanone was a powerful solvent and could then be dispersed in water with Lissapol N;* but aqueous dispersates with only 1% of cyclohexanone caused the insects to writhe so much that counts could not be taken.

A number of intact mounted insects were exposed to contact sprays of the insecticides applied in a spray tower according to methods already described (5). A volume of 0.8 cc. of a 0.63% solution was applied to give an area dosage of 5 $\mu$gm./cm.$^2$ Since the surface area of the dewinged roach exposed to falling spray is 4 sq. cm., this dosage corresponded to 20 $\mu$gm. per individual. Counts were made for a total period of 1000 min., except in cases where complete arrest supervened after 300 min. or more.

## Results

The average rates of the heartbeat for the intact mounted insect and for the decapitated insect, and for both types when injected with 10 cu. mm. of corn oil, are shown in Table I. The course of the heartbeat for the most typical of the replicates is plotted in Fig. 1; here the time is plotted on a logarithmic scale in order to accentuate the important early stages. Whereas the intact insect maintains a constant rate for the observation period, decapitation induces a

* *Octyl cresol poly(8–10) ether alcohol, Imperial Chemical Industries.*

TABLE I

AVERAGE RATE OF HEART PULSATIONS, IN BEATS PER MINUTE, IN INTACT AND DECAPITATED *Periplaneta*, AND THE RESPONSE TO INJECTION WITH 10 CU. MM. OF CORN OIL

| | Three intact insects | | | | Three decapitated insects | | | |
|---|---|---|---|---|---|---|---|---|
| *Uninjected* | | | | | | | | |
| | | Time in minutes | | | Before decapitation | Minutes after decapitation | | |
| | | 1–10 | 10–20 | 20–100 | | 1–10 | 10–20 | 20–100 |
| 1. | — | 133 | 132 | 133 | 92 | 85 | 79 | 73 |
| 2. | — | 128 | 125 | 128 | 106 | 107 | 102 | 99 |
| 3. | — | 123 | 130 | 123 | 72 | 75 | 69 | 63 |
| Av. | — | 128 | 129 | 128 | 90 | 89 | 83 | 78 |
| *Injected with corn oil* | | | | | | | | |
| | Pre-injection | Minutes after injection | | | Pre-injection | Minutes after injection | | |
| | | 1–10 | 10–20 | 20–100 | | 1–10 | 10–20 | 20–100 |
| 1. | 121 | 117 | 122 | 121 | 84 | 97 | 88 | 81 |
| 2. | 116 | 124 | 125 | 108 | 99 | 89 | 82 | 85 |
| 3. | 107 | 108 | 113 | 105 | 78 | 74 | 77 | 81 |
| Av. | 115 | 116 | 120 | 111 | 87 | 87 | 82 | 82 |

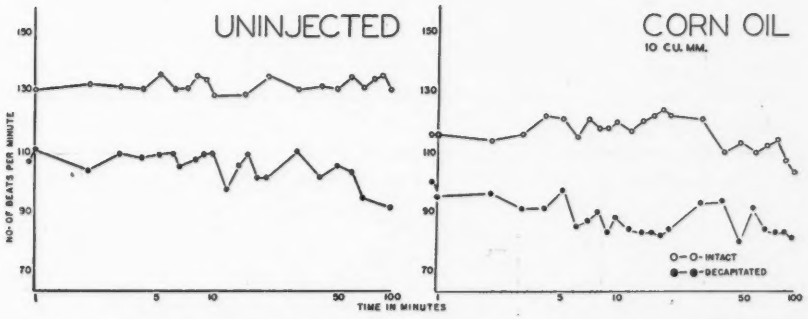


FIG. 1.—Effect of the injection of 10 cu. mm. of corn oil on the rate of heart pulsation of *Periplaneta*.

gradual decrease in the rate and amplitude of the pulsations. The injection of corn oil may be seen to have very little, if any, effect on the heartbeat of the intact or the decapitated cockroach.

The results of injecting certain of the chlorinated hydrocarbon insecticides are shown in Table II and Fig. 2. It is apparent that DDT at these dosages has virtually no effect on the heartbeat of the intact or the decapitated insect. The principal effect of injecting technical chlordane, lindane, or toxaphene is an irregularity in the pulsation rate of both the intact and decapitated insects. With these insecticides there is a tendency to an increase in the average rate of heartbeat, but the variability of the material does not allow acceleration to be claimed. When the injected insects finally died, the heart

TABLE II

AVERAGE RATE OF HEART PULSATIONS, IN BEATS PER MINUTE, IN INTACT AND DECAPITATED *Periplaneta* INJECTED WITH 80 μGM. OF CHLORINATED HYDROCARBON INSECTICIDES IN CORN OIL

| | Three intact insects | | | | Three decapitated insects | | | |
|---|---|---|---|---|---|---|---|---|
| | Pre-injection | Minutes after injection | | | Pre-injection | Minutes after injection | | |
| | | 1–10 | 10–20 | 20–100 | | 1–10 | 10–20 | 20–100 |
| *DDT* | | | | | | | | |
| 1. | 116 | 124 | 125 | 121 | 114 | 111 | 117 | 111 |
| 2. | 101 | 108 | 113 | 101 | 84 | 112 | 104 | 96 |
| 3. | 96 | 91 | 92 | 81 | 109 | 101 | 89 | 87 |
| Av. | 104 | 108 | 111 | 101 | 102 | 108 | 103 | 98 |
| *Chlordane (refined from technical product)* | | | | | | | | |
| 1. | 91 | 101 | 107 | 107 | 71 | 85 | 95 | 88 |
| 2. | 81 | 86 | 91 | 80 | 106 | 109 | 116 | 110 |
| 3. | 121 | 127 | 130 | 120 | 107 | 106 | 110 | 109 |
| Av. | 98 | 105 | 109 | 102 | 95 | 100 | 107 | 102 |
| *Lindane* | | | | | | | | |
| 1. | 126 | 134 | 126 | 123 | 144 | 134 | 143 | 137 |
| 2. | 132 | 126 | 139 | 117 | 128 | 131 | 142 | 122 |
| 3. | 123 | 118 | 120 | 125 | 111 | 120 | 121 | 124 |
| Av. | 127 | 126 | 128 | 122 | 128 | 128 | 135 | 128 |
| *Toxaphene* | | | | | | | | |
| 1. | 129 | 143 | 140 | 126 | 125 | 117 | 118 | 112 |
| 2. | 105 | 102 | 106 | 105 | 149 | 156 | 158 | 148 |
| 3. | 77 | 90 | 96 | 88 | 115 | 119 | 117 | 116 |
| Av. | 104 | 112 | 114 | 106 | 130 | 131 | 131 | 125 |

stopped in diastole in cases where DDT or chlordane had been employed; with toxaphene the systolic period of each beat became prolonged and the heart finally stopped in systole.

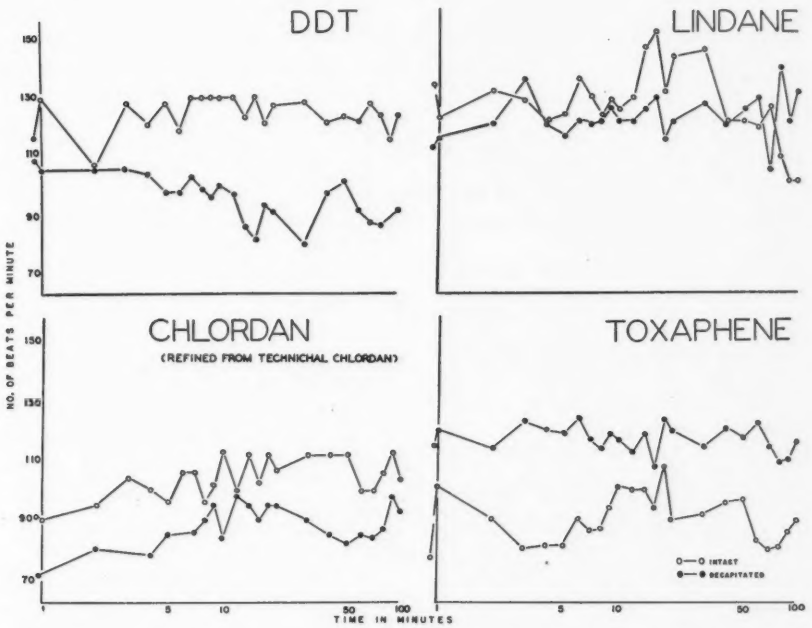


FIG. 2.—Effect of certain chlorinated hydrocarbons on the rate of heart pulsation of *Periplaneta*.

The effects of the dinitro compounds on the heartbeat are shown in Table III and Fig. 3. Both DNOC and DNCHP eventually arrest the heart pulsations, there being considerable variation in the time to stoppage. In nearly all cases a significant increase in pulsation rate develops, the peak occurring from 10 to 50 min. after injection. During this period of increase, the posterior segments of the heart may show temporary stoppages in diastole.

The effects of the injection of parathion, Lethane 60, and rotenone are shown in Fig. 4. Parathion causes an increase in pulsation rate, which develops more gradually in the decapitated roach. The heart eventually stops in systole. Lethane 60 causes an initial slight acceleration of the heart in the first 5 or 10 min. but thereafter the rate gradually decreases. There is also a decrease in the amplitude of the systolic contraction, and the heart eventually stops in diastole. The effect of rotenone on the heart pulsations is decisive. In the intact insect, the rate declines steadily and may cease within 20 min., this decline sets in later and is more gradual in the decapitated roach. The

anterior segments are the first to cease beating; and a decrease in amplitude precedes the final arrest in diastole.

TABLE III

AVERAGE RATE OF HEART PULSATIONS, IN BEATS PER MINUTE, IN INTACT AND DECAPITATED *Periplaneta* INJECTED WITH 80 μGM. OF CERTAIN ORGANIC INSECTICIDES IN CORN OIL

| | Three intact insects | | | | Three decapitated insects | | | |
|---|---|---|---|---|---|---|---|---|
| | Pre-injection | Minutes after injection | | | Pre-injection | Minutes after injection | | |
| | | 1–10 | 10–20 | 20–100 | | 1–10 | 10–20 | 20–100 |
| *Parathion* | | | | | | | | |
| 1. | 113 | 119 | 121 | 114 | 114 | 124 | 116 | 128 |
| 2. | 128 | 144 | 147 | 141 | 128 | 124 | 129 | 136 |
| 3. | 128 | 130 | 134 | 131 | 101 | 111 | 105 | 125 |
| Av. | 123 | 131 | 134 | 129 | 114 | 120 | 117 | 130 |
| *Lethane 60* | | | | | | | | |
| 1. | 120 | 129 | 127 | 118 | 112 | 137 | 123 | 120 |
| 2. | 126 | 130 | 131 | 128 | 106 | 100 | 100 | 100 |
| 3. | 119 | 129 | 121 | 119 | 106 | 115 | 112 | 105 |
| Av. | 122 | 129 | 126 | 122 | 108 | 117 | 112 | 108 |
| *Rotenone* | | | | | | | | |
| 1. | 128 | 127 | 101 | 40m* | 137 | 125 | 99 | 82 |
| 2. | 117 | 113 | 74 | 62 | 124 | 121 | 95 | 77 |
| 3. | 115 | 119 | 18m | — | 125 | 120 | 93 | 82 |
| Av. | 120 | 120 | 87 | — | 129 | 122 | 96 | 80 |
| *DNOC* | | | | | | | | |
| 1. | 104 | 117 | 118 | 92m | 114 | 122 | 104 | 30m |
| 2. | 106 | 106 | 109 | 80m | 108 | 90 | 107 | 131 |
| 3. | 103 | 3m | — | — | 76 | 91 | 88 | 60m |
| Av. | 104 | 111 | 114 | — | 99 | 101 | 100 | — |
| *DNCHP* | | | | | | | | |
| 1. | 92 | 92 | 105 | 30m | 72 | 102 | 111 | 94 |
| 2. | 73 | 81 | 78 | 89 | 97 | 96 | 12m | — |
| 3. | 103 | 105 | 105 | 91 | 72 | 95 | 99 | 40m |
| Av. | 89 | 93 | 96 | 90 | 80 | 98 | 100 | — |

* *The expression 40m etc. denotes that the heart stopped beating 40 min. etc. after injection.*

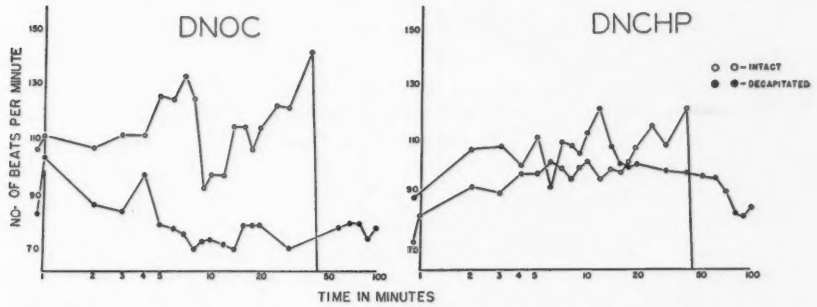


FIG. 3.—Effect of certain dinitro compounds on the rate of heart pulsation of *Periplaneta*.

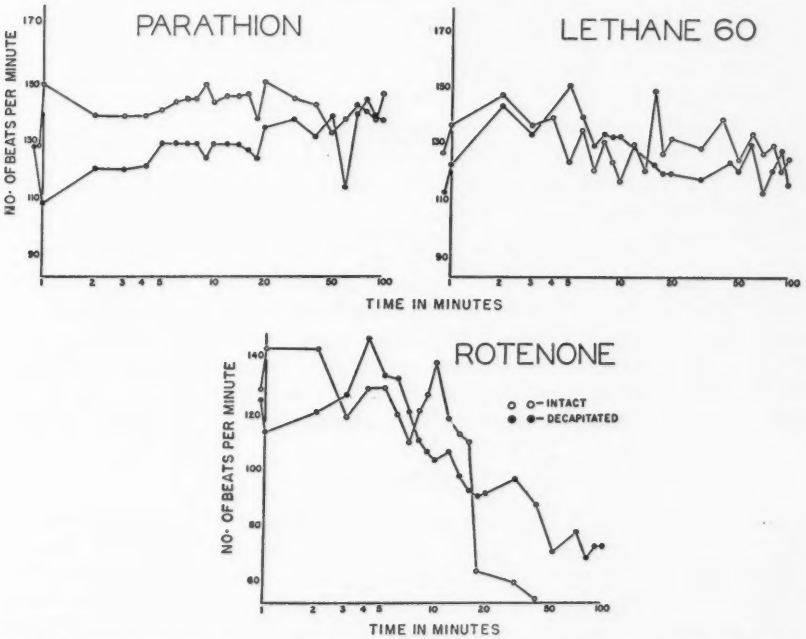


FIG. 4.—Effect of Lethane 60, parathion, and rotenone on the rate of heart pulsation of *Periplaneta*.

When the benzene–kerosene solvent was sprayed directly on the intact insects, the heartbeat was significantly depressed, before returning to the normal rate 10–15 min. later (Fig. 5). This effect was presumably due to a temporary narcosis caused by the spray liquid and its vapor. When DDT or chlordane was dissolved in the spray, although the rate was found to return to the normal after the initial narcotic effect, after 200–500 min. the rate steadily decreased as the insects reached advanced stages of poisoning. With

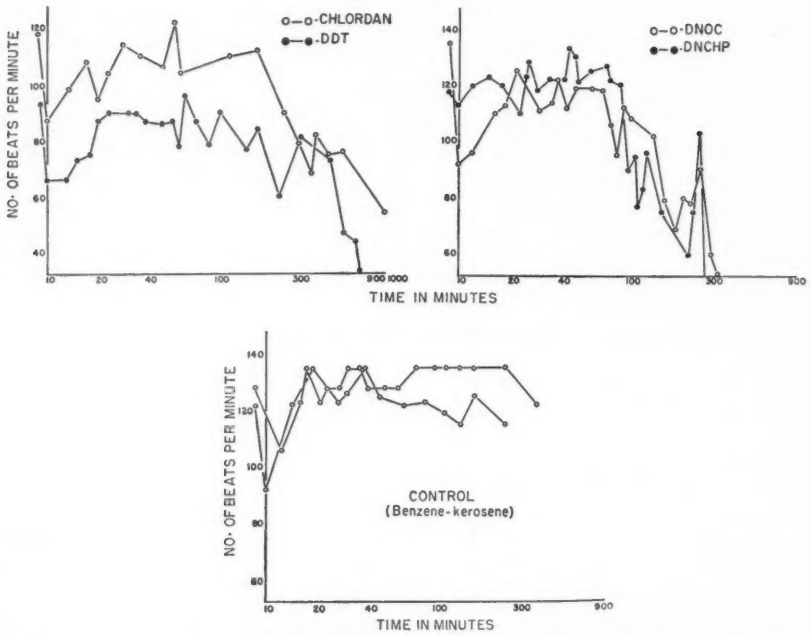


FIG. 5.—Effect of certain insecticides, applied by spraying in benzene–kerosene solution, on the heartbeat of *Periplaneta*.

DNOC and DNCHP this decrease was more abrupt, commencing 60 min. after the application; a curious characteristic was the sudden but ephemeral rally in rate which occurred immediately before the complete diastolic arrest.

## Discussion

The effect of decapitation in reducing the amplitude and progressively decreasing the frequency of the heartbeat may be attributed to the removal of the tonic effect of the supraesophageal ganglion. Decapitation also renders the heart rate less liable to irregularities that may be caused by insecticides through their exciting effect on the central nervous system (e.g. lindane, Fig. 2). The heart of the decapitated insect is slower to respond, and the response is more gradual (e.g. rotenone, Fig. 4).

The slight effect of DDT on the heartbeat of the intact and of the decapitated *Periplaneta* is in accordance with the results of Davenport (4), who found this insecticide to have no effect on the isolated heart of the orthopteran *Stenopelmatus*. A similar situation obtains with the other chlorinated hydrocarbons tested, which in most cases had a slightly stimulating and unsettling effect on the heart of the intact animal and appeared to counteract the normal deceleration of the heartbeat in the decapitated animal.

The dinitro compounds DNOC and DNCHP have a definite effect on heartbeat, characterized by eventual arrest, nearly always preceded by stimulation. It was observed that temporary stoppages frequently occurred after short periods where the pulsation rate exceeded 150 beats per minute. The heart of the decapitated animal was found to be much less sensitive, and in some cases was not stimulated at all by the dinitro compound. The accelerating effect may therefore be related to the general stimulation of the nervous system, which is indicated by the material increase in respiratory rate of roaches poisoned with dinitro compounds (5). However, a rise in respiratory activity does not necessarily involve an increase in heart rate; the chlorinated hydrocarbons are strong respiratory stimulants while having little effect on the heart.

The finding that parathion increases heart rate in the intact insect bears out the discovery of Krijgsman and Krijgsman (6) that acetylcholine and anticholinesterases are accelerators of the isolated heart of *Periplaneta*. The slightly stimulating effect of Lethane 60, found in five out of six individuals tested, is presumably attributable to the low dosage applied; when injections of 100 μgm. per individual, or more, were applied to *Periplaneta*, they proved to be depressant. This is in agreement with Coon (3), who found that a dose of 125 μgm. per individual of Lethane 384 applied by contact had a markedly depressing effect on the heart rate, and of Yeager, Hager, and Straley (9) who established that the isolated heart of *Blatta* was retarded by aliphatic thiocyanates in direct proportion to the concentration applied.

The results obtained with rotenone are in essential agreement with those of Tischler (8), who found that the application of derris dusts to *Bombyx* larvae caused a marked decrease in the heartbeat which appeared even when the caterpillars were still active. The depressant action of rotenone on the decapitated roach shows that this effect is largely independent of the general activity of the insect.

## Conclusions

The chlorinated hydrocarbon insecticides have little effect upon the rate of heart pulsation when injected into the American roach. The dinitro compounds stimulate the pulsation rate in the intact insect, and the peak of stimulation is followed by total failure of the heart. Rotenone steadily depresses the rate of heartbeat until it ceases altogether.

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# SOME OBSERVATIONS OF THE NUMBER OF BLACK FLIES (DIPTERA, SIMULIIDAE) LANDING ON COLORED CLOTHS[1]

By D. M. Davies[2]


## Abstract

The number of black flies alighting on cloths of different colors, each tested with a control of black cloth, varied with the intensity of the light reflected from the cloth and to a lesser extent with the wavelength. Dark blue was the color most frequented followed by dark brown, black, dark green, dark red, medium gray, and white.


## Introduction

Insects have long been known to respond differently to light of various colors. Many are attracted more by violet and ultraviolet than by light of other wavelengths (7). Weiss (5, 6), studying many species of insects, found that if the relative energy of different wavelengths was made uniform, the greatest response occurred at 365 millimicrons (ultraviolet) with a smaller peak at 492 m$\mu$ (blue-green). By changing the intensity of each color, the response of the insect could be made uniform from one color to another. He states in conclusion that "of the two inseparable constituents, wavelength and intensity, the latter seems to be the most important in producing reactions."

It is apparent from experiments with certain mosquitoes and other diptera (1, 2, 4) that many alight more often on dark materials and select blue and violet surfaces rather than those of other colors. However, all diptera do not react in this way (2, 3).

Qualitative observations by the author suggested that black flies land more frequently on dark than on light clothing when it is being worn. However, it was observed also that few flies landed on dark cloth that was off the body, even when the cloth had been soaked in human and artificial perspiration. It seemed important, therefore, to determine the number of black flies landing on black and white cloths and also on those of different colors while they were on the human body.

## Methods

The study proceeded from June 27 to July 5, 1947, in Algonquin Park, Ontario. Tests were made on cloths having the following Munsell color notations: dark blue (8.5 PB 1.8/3.0), dark brown (1.5 YR 2.0/1.8), black N 1.2/yellowish), dark green (1BG 2.0/1), dark red (6 R 2.3/9), medium gray (N 5.1/), and white (N9$^+$/), the black being a control in all tests.

In each test the black cloth and one of the other colors were laid side by side on the observer's leg, the black being the closer to the body in approxi-

[1] *Manuscript received November 7, 1950.*

*Supported by grant from Province of Ontario on recommendation of Research Council of Ontario.*

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mately half the tests. The black flies alighting on the two adjacent 6 in. square areas of these cloths, shaded from the direct sunlight, were counted simultaneously during intervals of two minutes.

The intensity of the total light reflected from each cloth (the reflectance) was measured with a "Weston Master" exposure meter (model 715). The reading of this meter represents approximately a visual intensity factor because the meter has a light sensitivity similar to that of the human eye and is more sensitive to the blue region of the spectrum. The change in intensity of reflected light from 400-750 m$\mu$ was recorded for each cloth with a recording spectrophotometer* using a standardized opaque, white vitrolite glass plate as control. For lower wavelengths (230-405 m$\mu$) reflectances from the cloths were compared by a "Bellingham and Stanley" spectrograph, using as the light source a mercury-arc, direct current, quartzite lamp from which a high proportion of the radiation was ultraviolet. The spectrum of the reflected light was recorded in a 10 min. exposure on an orthochromatic, photographic plate.

## Results

The identification of black flies, collected immediately before or after the tests, indicated that over 90% were *Simulium venustum* Say.

A black cloth was used as a control in each test as the tests were made on different days and the number and activity of the flies at the station might differ. However, when two cloths are placed side by side it may be that the color of one cloth may influence the number landing on the other. It has seemed advisable, therefore, to consider the number of flies landing on the test cloth as a percentage of the total number landing on both it and the control (Table I) rather than to compare the number landing on the color to

TABLE I

A COMPARISON OF THE NUMBER OF BLACK FLIES LANDING ON COLORED CLOTHS AND THE REFLECTION OF LIGHT FROM THE CLOTHS

| Color | Number of tests | Total number landing on cloths | | Landing percentage | Reflectance ratio | |
|---|---|---|---|---|---|---|
| | | Colored | Black control | $\left(\frac{\text{Color}}{\text{Total}} \times 100\right)$ | Exposure meter $\frac{\text{(color}}{\text{black)}}$ | Spectrophotometer $\frac{\text{(color}}{\text{black)}}$ |
| Dark blue | 12 | 346 | 82 | 81.3 | 2.1 | 2.4 |
| " brown | 10 | 81 | 34 | 70.5 | 1.6 | 2.4 |
| " green | 7 | 44 | 54 | 44.9 | 1.3 | 2.1 |
| " red | 10 | 19 | 43 | 30.6 | 1.5 | 6.6 |
| Med. gray | 13 | 86 | 231 | 27.1 | 8.2 | 10.4 |
| White | 26 | 52 | 634 | 7.6 | 26.5 | 37.4 |
| Black | 78 | — | — | — | 1.0 | 1.0 |

* *Measurements were made through the courtesy of the Photometry and Colorimetry Laboratories, Optics Section, National Research Council of Canada.*

the number landing on the black control. This is referred to as the *landing percentage*. The data on which the percentages are based are plotted in Fig. 1. The square roots of the numbers were used in the figure, as is usually done with this type of data, to make the variance more uniform.

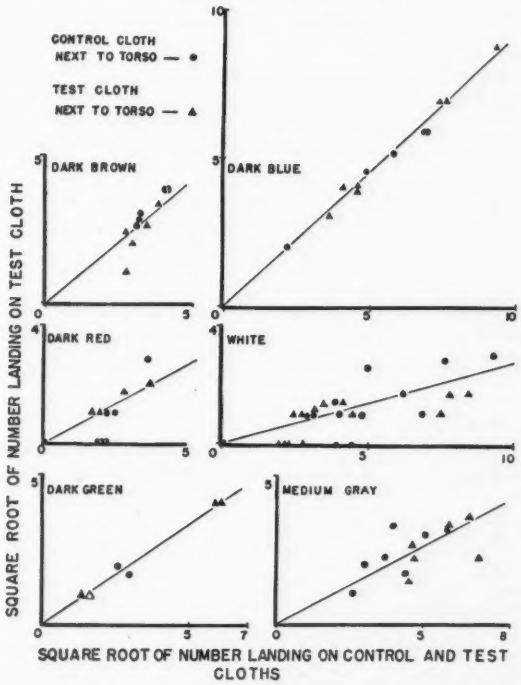


FIG. 1.—The square root of the number of black flies landing on a 6 in. square of each colored test cloth plotted against the square root of the total number landing on adjacent 6 in. squares of the test cloth and the black control cloth during an interval of two minutes. The straight line passes from the origin through the arithmetic mean.

The lowest *landing percentage* was obtained with white cloth and the highest with the dark materials, medium gray being intermediate (Table I). The *landing percentage* for the dark materials varied from color to color decreasing in this order: blue, brown, green, and red. In the tests with the black and green cloths the number landing on both was about equal, with that for the black being a little higher.

The total reflectances of the colored cloths, as measured by the exposure meter and as calculated from the areas under the spectrophotometric curves (correcting for scale differences), were each divided by that for the black cloth recorded at the same time (Table I). These ratios are referred to as the *reflectance ratios*. The *reflectance ratios* derived from the spectrophotometric readings are only a little higher than those derived from the readings

of the exposure meter, except in the case of the red cloth which is over four times the value. The cloths with the lowest *landing percentages*, white and gray, had the highest *reflectance ratios*. Little difference was found between the *reflectance ratios* of the various dark cloths (except in the case of the ratio of the spectrophotometric readings for the red which also had the lowest *landing percentage* of the dark cloths), although the *landing percentages* were distinctly different.

The intensities of all wavelengths of light reflected from the black control cloth, as measured by the spectrophotometer, are almost equal (Fig. 2). The

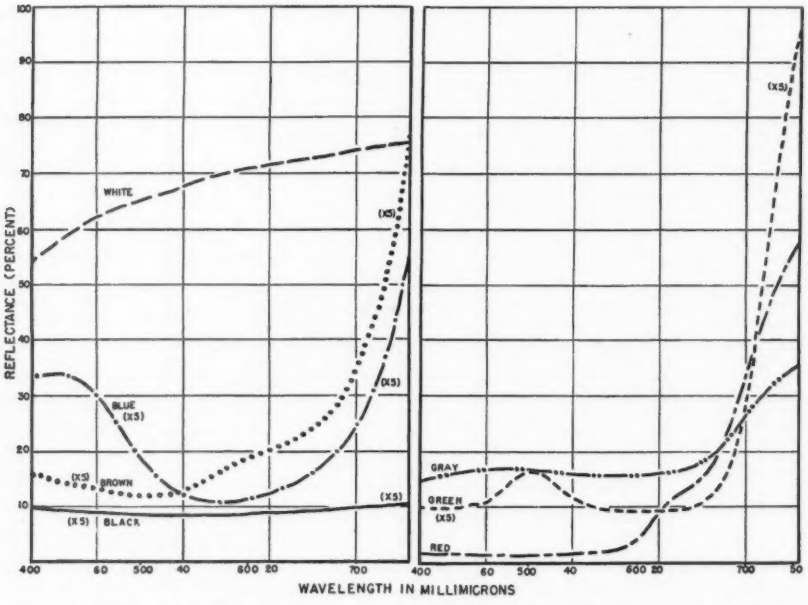


FIG. 2.—The variation in intensity with wavelength for each cloth as measured by the recording spectrophotometer. The values for the blue, brown, black, and green were multiplied by five so that all the curves could be compared on the same graphs.

reflectances of the gray and the other dark cloths have a high red component (Fig. 2) but the blue cloth has also a high blue component. The curve for white rises gradually in intensity from 400-750 m$\mu$.

If the intensity of reflected light from 400-460 m$\mu$ for the dark cloths, as calculated from the areas under the curves, is considered (Table II) the values decrease from blue, through brown, green, and black, to red. This is the reverse of the sequence of values for the *landing percentages*, except for green and black which are almost equal in reflectance and in the number of flies landing on them. The white and gray cloths with low *landing percentages* have much higher reflectances than the other cloths in this range of wavelengths.

PLATE I

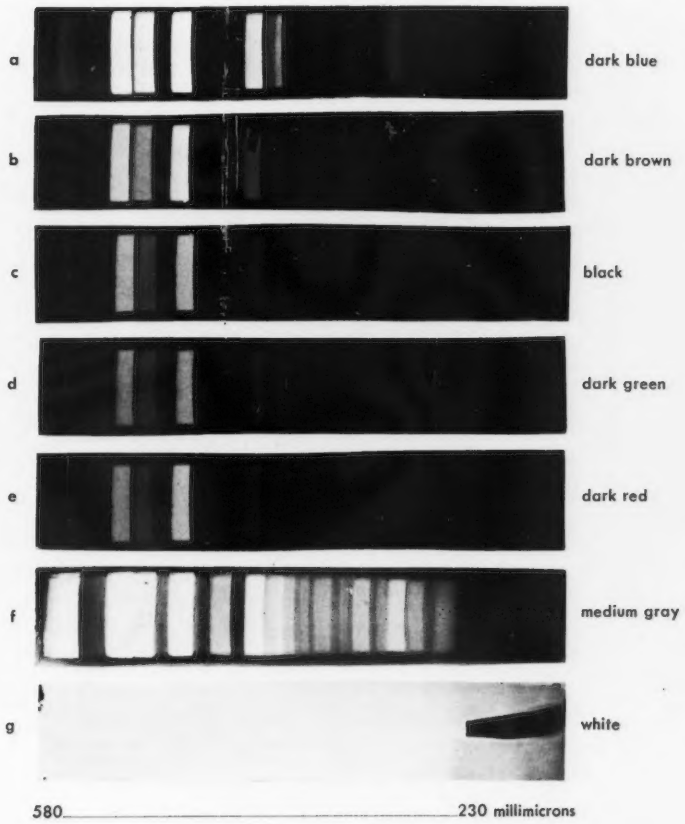


FIG. 3.—Spectrograms of the reflection from colored cloths exposed for 10 min. to the light of a mercury-arc, quartzite lamp.

PLATE II

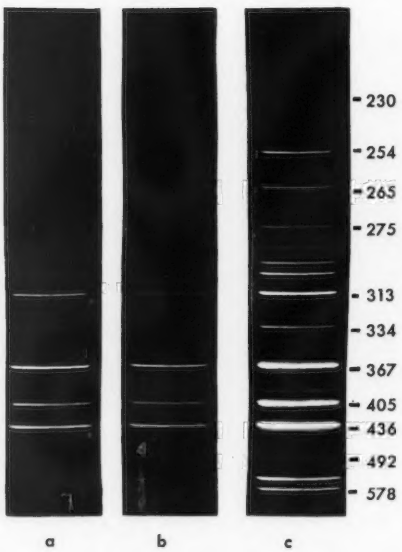


FIG. 4.—Spectrograms as in Fig. 3 but with the slit narrowed to show the individual bands in the white (*c*) for comparison with the blue (*a*) and black (*b*) and for labelling the bands.

TABLE II

AREAS UNDER CURVES FOR EACH WAVELENGTH INTERVAL AS AN INDEX OF INTENSITY OF REFLECTED LIGHT (EACH VALUE IS CORRECTED FOR INTERVAL AND SCALE DIFFERENCES AND DIVIDED BY THE VALUE FOR THE BLACK CONTROL)

| Color | Wavelength in mμ | | | | |
|---|---|---|---|---|---|
| | 400-460 | 460-540 | 540-620 | 620-700 | 700-750 |
| Dark blue | 3.59 | 2.30 | 1.33 | 1.78 | 3.62 |
| Dark brown | 1.57 | 1.42 | 1.95 | 2.60 | 4.95 |
| Black | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Dark green | 1.08 | 1.63 | 1.14 | 1.38 | 6.20 |
| Dark red | 0.774 | 0.774 | 1.86 | 9.23 | 23.2 |
| Medium gray | 8.68 | 9.64 | 9.28 | 10.2 | 15.5 |
| White | 31.9 | 37.6 | 40.9 | 38.3 | 36.8 |

The reflectance of all wavelengths from 230-405 mμ was greater for the white and gray cloths than for the dark cloths as shown by the spectrograms (Figs. 3*a-g*, 4*a-c*). The spectrograms for the dark cloths (Figs. 3*a-e*, 4*a*, *b*) record the fact that the greatest reflectance of ultraviolet and violet wavelengths occurred with blue cloth; brown, black, red, and green cloths showed respectively decreasing reflectances in these wavelengths. The dark cloths with the highest reflectances of ultraviolet and violet wavelengths had the highest *landing percentages*.

A few black flies landed on each of the cloths but they frequented some more than others. The number of black flies alighting on a cloth was influenced more by the intensity than by the wavelength of the reflected light. Few flies landed if the total intensity of the light reflected from the cloths was high and more flies landed when intensity was low. When the total intensity of the reflected light was similar the highest *landing percentages* were obtained with cloths having the highest intensity in the blue to ultraviolet region of the reflected light.

Thus the wearing of white clothing would be desirable where black flies of the species *S. venustum* are common. On the other hand dark clothing, particularly blue, would be the least desirable.

## Acknowledgments

Sincere thanks are extended to Dr. H. B. Speakman, Director, Ontario Research Foundation, who encouraged and provided the opportunity for this research. The interest and counsel of Prof. F. P. Ide, University of Toronto, Dr. A. M. Fallis, Ontario Research Foundation, and Dr. D. H. Hamly of the University and the statistical advice of Dr. D. B. Delury of the Foundation is gratefully acknowledged. I wish to thank Prof. K. B. Jackson of the University for providing facilities and advice in spectroanalysis and Prof. R. R. Langford, Ontario Fisheries Research Laboratories, for facilities in studying the black flies during the summer.

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